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EFFECTS OF AROMATIC CONCENTRATION ON METHANE FERMENTATION

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ABSTRACT

Title: Effects of Aromatic Concentration on Methane Fermentation

The anaerobic biodegradability and toxicity of fourteen aromatic compounds were evaluated over a wide range of concentrations using a serum bottle technique. Benzene, toluene, and all three isomers of xylene were not significantly degraded to methane in a phenol-enriched culture. Complete degradation of 1000 mg/L phenol, 800 mg/L catechol, 100 mg/L 2-NP, 100 mg/L 3-NP, and 100 mg/L 4-NP was observed within two months while depletion of 100 mg/L resorcinol and 1000 mg/L hydroquinone required more than six and eight months incubation, respectively. None of the three isomers of chlorophenol were degraded in the phenol-enriched culture. Batch toxicity assay revealed that the phenol-enriched culture was more susceptible to inhibition caused by substituted phenols than the acetate-enriched culture. In general, the inhibitory effects on both phenol degradation and acetate utilization did not vary significantly with the isomer but rather with the substituted group. The degree of inhibition was in the order of nitrophenols > chlorophenols > hydroxyphenols. The Haldane inhibition model was used to fit experimental data from phenol and catechol. The inhibition of phenol degradation by chlorophenols, resorcinol, and hydroquinone was described rather well by a Monod-type, noncompetitive model.

Descriptors: Anaerobic Digestion, Fermentation, Aromatic Compounds

Identifiers: Kinetics, Inhibition, Methane, Phenols, Acetate

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I. INTRODUCTION

Objective

The overall objective of the proposed research was to evaluate the kinetics of biodegradation and inhibition of a group of aromatic compounds under anaerobic conditions. The effect of concentration of these compounds on various groups of bacteria in methanogenic cultures were studied. The relationship between the molecular structure of model aromatic compounds and their respective rate of degradation and inhibition were assessed.

Fifteen aromatic compounds including benzene, toluene, phenol, and the ortho, meta, and para isomers of chlorophenol, nitrophenol, hydroxyphenol (catechol, resorcinol, and hydroquinone) as well as xylene were selected as the model compounds. A batch procedure was used as a tool for evaluating the biodegradability and toxicity of these compounds. A phenol-enriched culture and an acetate-enriched culture were used as the inoculum for batch studies. These cultures were maintained in two continuous flow fermentors operated at a liquid retention time of 30 days. Batch test data were analyzed using modified Monod kinetic equations for substrate utilization and inhibition.

Background

Aromatic compounds and many of their derivatives are widely used in a number of industrial operations such as the manufacture of chemical solvents, pesticide, polymers, explosives, and many other products of everyday use. Many aromatic derivatives can pose a health hazard and have been designated as priority pollutants by the U.S. Environmental Protection Agency. Most are toxic at relatively high concentrations and some are carcinogenic at exceedingly low concentrations (Miller, 1970, Wallcave et al. 1971; Dean,1978). Consequently, the dissemination of these materials into the

environment, accidental or otherwise, is a problem of grave concern. The fate of these compounds in the environment is, therefore, of great interest. Anaerobic biodegradation is one process which affects the fate of organic contaminants in the environment. However, relatively little is known about the biodegradation of aromatic compounds under anaerobic conditions. Since anaerobic conditions commonly exist in soils and sediments and anaerobic treatment of sewage sludge and various type of wastewaters is an important methodology for pollution control, this study encompassed a detailed study on the effect of concentration of model aromatic compounds on their biodegradation and inhibition under anaerobic conditions.

Literature Review

Evans (1977) reviewed the biochemistry of anaerobic bacterial catabolism of aromatic compounds while Sleat and Robinson (1984) discussed the microbiology of anaerobic aromatic degradation. Evidence that the anaerobic breakdown of the aromatic ring is different and quite distinct from the aerobic pathways was provided by early studies using benzoate as the substrate through photometabism (Hegeman, 1967; Dutton and Evans, 1968; Guyer and Hegeman, 1969; Dutton and Evans, 1969), nitrate respiration (Oshima, 1965; Taylor, et al., 1970; Taylor and Heeb, 1972; Williams and Evans, 1973; Bakker, 1977), and methane fermentation (Tarvin and Buswell, 1934; Clark and Fina, 1952; Fina and Fiskin, 1960; Nottingham and Hungate, 1969; Keith, 1972; Keith et al., 1978), respectively. It has now been established that in all of the above three sets of conditions investigated where aromatic compounds are degraded in the absence of oxygen, dissimilation occurs first by reduction of the ring, then by a reductive cleavage of the ring to aliphatic acids (Figures 1-3), despite the different pathways proposed by different investigators. The utilization

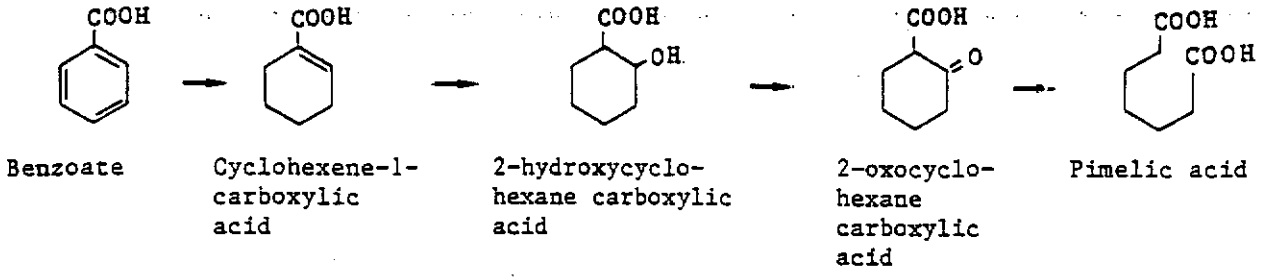

Figure 1. Reductive Pathway for the Degradation of Benzoic Acid by a Rhodospeudomonas sp. (Photometabolism, Dutton and Evans, 1968)

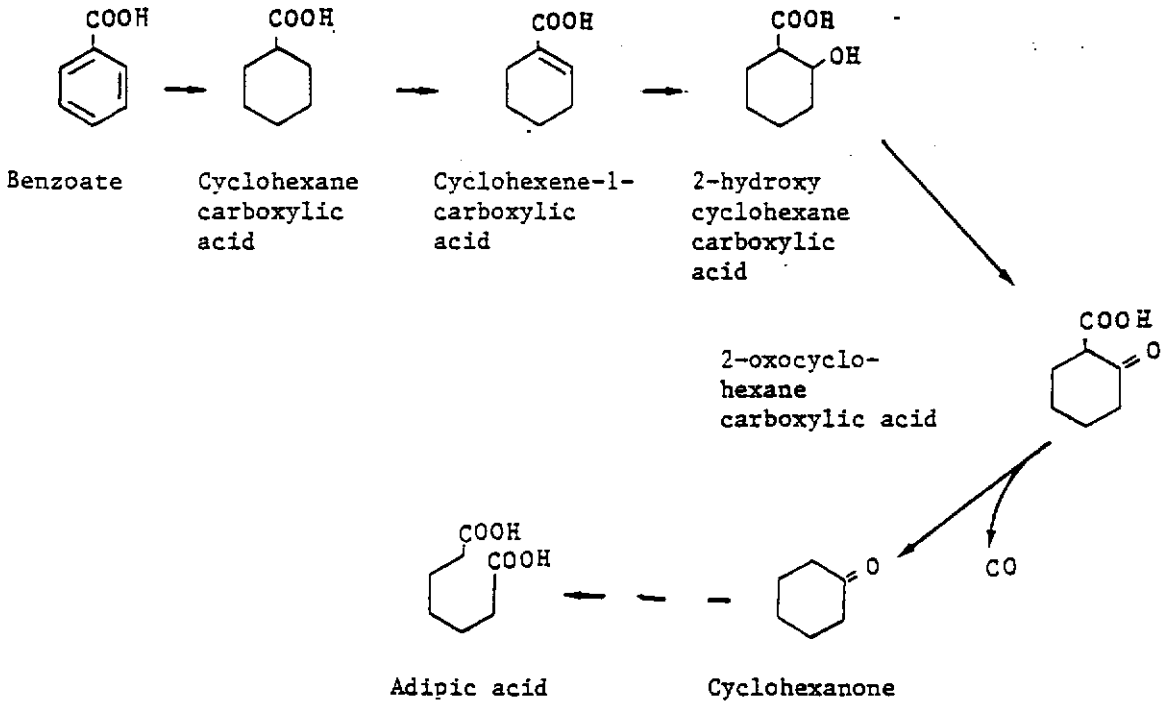

Figure 2. Reductive Pathway for the Degradation of Benzoate by Nitrate-respiring Bacteria. (Williams and Evans, 1975)

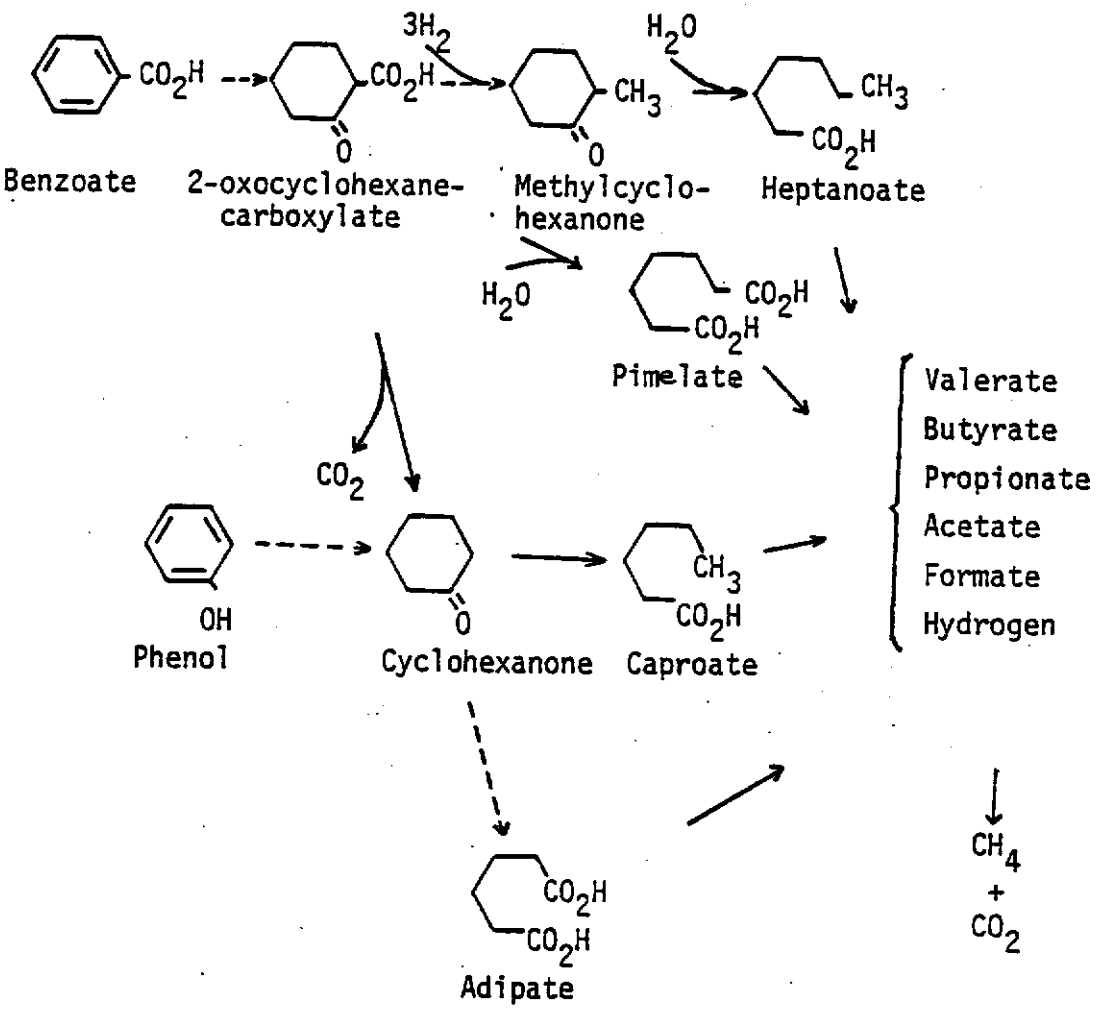

Figure 3. Probable Pathways in the Fermentation of Benzoate and Phenol by Adapted Bacterial Consortia from a Variety of Methanogenic Ecosystems (Evans, 1977)

of any of these pathways is determined by the bacterial species and environmental factors.

The anaerobic metabolism of substituted aromatic compounds requires transformation steps to initiate degradation. Substituents are generally removed to produce benzoate or phenol before ring reduction and ring cleavage occur. Recent studies on a range of substituted benzoates and phenols in pure cultures or in methanogenic enrichments indicated that dehalogenation (suflita et al., 1982; Boyd et al., 1983; Horowitz et al., 1983; Boyd and Shelton, 1984), demethoxylation (Boyd, et al., 1983; Kaiser and Hanselmann, 1982; Balba et al., 1979; Bache and Pfenning, 1981), demethylation (Grbic-Galic and Pat-Polasko, 1985; Young and Rivera, 1985), decarboxylation (Tschech and Schink, 1985; Tshech and Schink, 1986; Samain et al., 1986), and dehydroxylation (Szewzyk et al., 1985; Tschech and Schink, 1986; Patel et al., 1981) took place prior to ring fission. During these transformations the methoxy substituents were transformed to hydroxy groups while the methyl, chloro, and hydroxy substituents were removed from the ring yielding benzoate and phenol as the methabolic intermediates before entering a common anaerobic degradation scheme (Figure 4). However, much of the evidence for the above reactions originate from earlier studies carried out with rumen and gut microflora (Booth and Williams, 1963; Scheline, 1968; Scheline, 1973; Peppercorn and Goldman, 1971). Recent work by Gabic-Galic and Vogel (1987) indicated that benzene and toluene were transformed to phenol, cresols, or benzyl alcohol via initial oxidation by ring hydroxylation or methyl oxidation in mixed methanogenic cultures derived from ferulic acid-degrading sewage sludge enrichments.

There are only a few reported studies on the kinetics of degradation or transformation of aromatic compounds under methanogenic conditions. Dehalogenation of 3-chlorobenzoate, 3,5-dichlorobenzoate, and 4-amino-3,5-

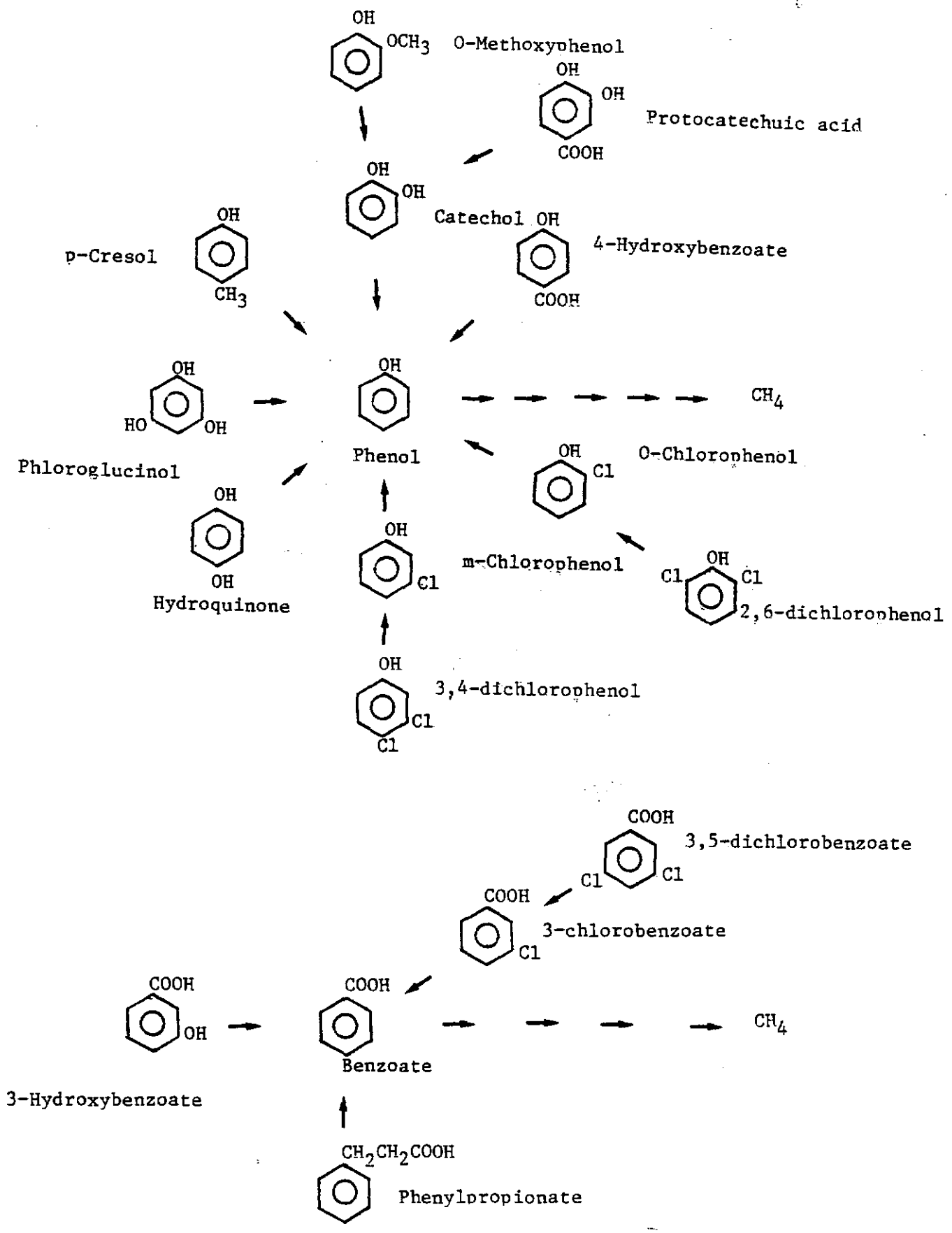

Figure 4. Summary of Anaerobic Biotransformation of Substituted Phenols and Benzoates under Methanogenic Conditions

dichlorobenzoate in methanogenic environments was described by Michaelis-Menten kinetics (Suflita, et al., 1983) while a modified Monod kinetic model which incorporated substrate inhibition terms was used to evaluate the degradation of phenol in methanogenic cultures (Suidan, et al., 1986; Dwyer, et al., 1986).

The anaerobic fate of many aromatic compounds under methanogenic conditions has only recently been studied although Tarvin and Buswell (1934) showed that benzoate, phenylacetate, phenylpropionate, and cinnamate could be degraded to methane more than 50 years ago. Chmielowski and Wasilewski (1966) screened 18 phenols and found that nine of these were amenable to methanogenic fermentation. Recently, the anaerobic degradation of catechol (Healy and Young, 1978), several substituted phenols (Boyd et al., 1983; Fedorak and Hrudey, 1984; Wang et al., 1988), and chlorinated phenols (Boyd and Shelton, 1984) to methane have been reported. There is some direct evidence that lignin is susceptible to biological attack under anaerobic conditions (Murray, 1974; Odier and Monties, 1978) while Healy and Young (1979) demonstrated that a range of 11 simple aromatic lignin derivatives were biodegradable to methane and carbon dioxide. Furthermore, Horowitz et al., (1982) assessed the anaerobic biodegradability of a variety of substituted aromatic compounds by measuring net production of methane. Their studies revealed that the chloro and amino substituents rendered the benzoate and phenol persistent while the hydroxy, methoxy, and bromo groups generally facilitated benzoate degradation. The anaerobic degradation of a nitrogen containing aromatic compound, indole, to methane was recently reported by Wang et al. (1984). A further study by Berry et al. (1987) revealed that indole was metabolized via oxindole to methane.

Based their work on benzoate, Ferry and Wolfe (1976) showed that the

methanogenic fermentation of aromatic ring required the cooperation of several groups of bacteria and that the methanogenic bacteria serve only as the terminal organism of a metabolic food chain. Removal of intermediates by methanogens provides thermodynamically favorable conditions for the degradation of many aromatic compounds such as benzoate and phenol. In recent years a variety of morphological types of methanogenic bacteria have been isolated while only eight substrates, none of them aromatic, have been shown to be converted to methane by this unique group of bacteria (Balch et al., 1979; Smith et al., 1980; Wolfe and Higgins, 1979 ; Zeikus 1980).

Although previous work has shown that a number of aromatic compounds are biodegradable under anaerobic conditions, few studies have been conducted to assess the effect of concentration of such compounds on their biodegradation and toxicity. Batch bioassays have been employed to evaluate the response of methanogenic consortia to phenols (Fedorak and Hrudey, 1984; Pearson et al., 1980; Benjamin et al., 1984; Wang et al., 1988) and the aromatic constituents of coal conversion wastewaters (Blum et al., 1986). Furthermore, little is known about the kinetics of biodegradation and inhibition of aromatic compounds in anaerobic environments. In this study, the kinetics of degradation and inhibition of model compounds under methanogenic conditions were investigated using a phenol-enriched and an acetate-enriched cultures.

II. MATERIALS AND METHODS

Aromatic Compounds

Benzene, toluene, phenol, and the ortho, meta, and para isomers of chlorophenol, hydroxyphenol, nitrophenol, as well as xylene were evaluated in this study. Figure 5 represents the matrix of the model compounds selected for this study. These compounds were selected to evaluate the effects of both the position and the type of substituents involved in anaerobic degradation and inhibition. Thermodynamic considerations indicate that degradation of all of the selected compounds under methanogenic conditions is possible although homoacetic acid fermentation of several of these compounds is thermodynamically unfavorable (Tables 1 and 2). This hypothesis was tested in the study. Phenol and acetate were used as the biodegradable control compound in each biodegradability test to ensure viability of the culture inocula.

Batch Bioassay

A batch procedure was used for the biodegradation and inhibition studies. The procedure for conducting these tests involves the placement in a test serum bottle of (a) a microbial inoculum, (b) a nutrient and reducing medium , and (c) a measured quantity of the organic compounds to be tested.

Seed inoculum. Two different cultures were used as the inculum for serum bottle studies:

a. Phenol-enriched methanogenic culture. A phenol-enriched methanogenic culture had been developed in our laboratory for more than two years. This culture was maintained in a continuous-flow 14-L fermenter (Model MF-114, New Brunswick Scientific Co., Edison, NJ) on a synthetic feed containing 4000 mg/l phenol and 5 mg/l of each of the following monosubstituted phenols: o-cresol, m-cresol, p-cresol, catechol, resorcinol, and hydroquinone. The organic

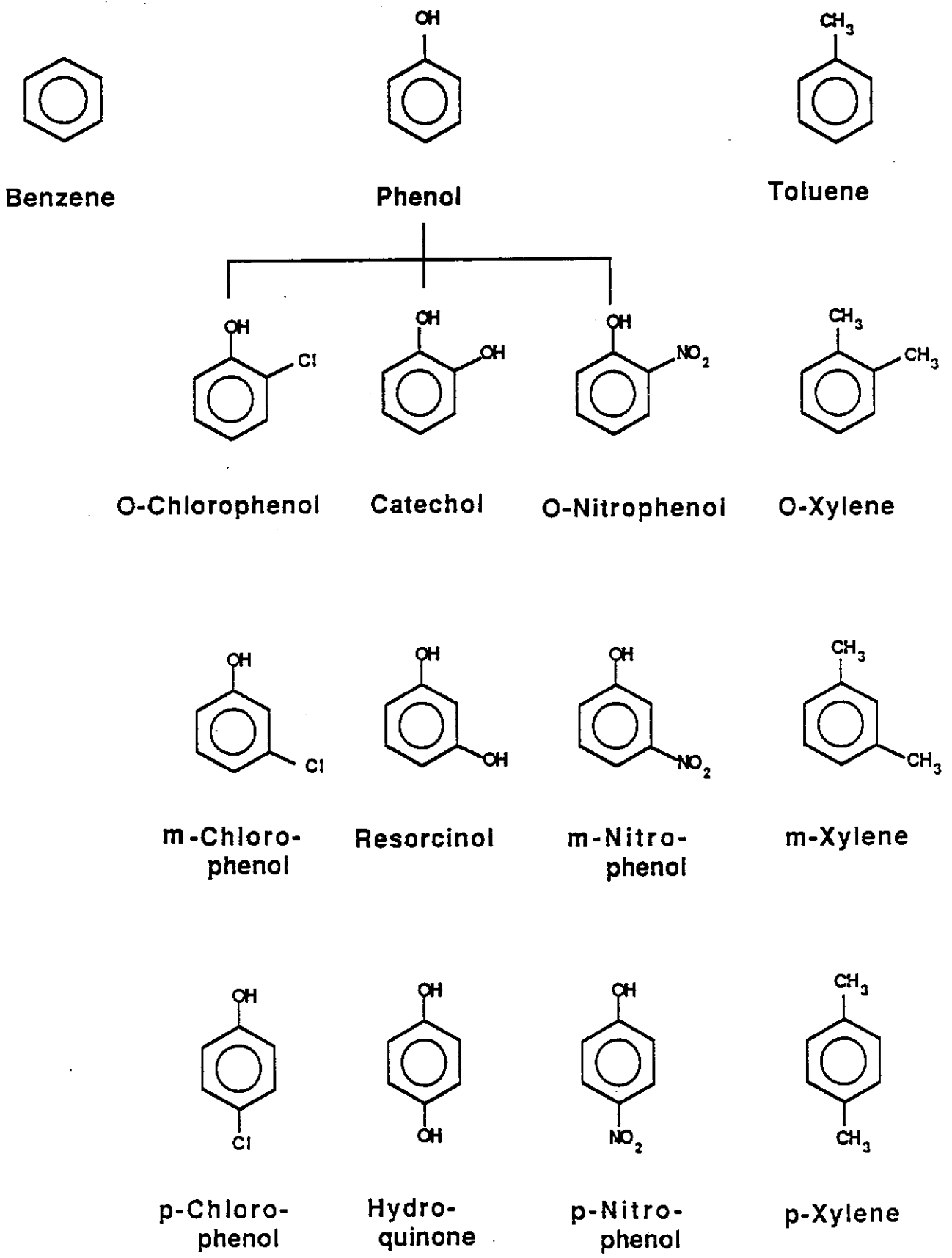

Figure 5. Aromatic Compounds used in Study

Table 1 Standard Free Energies of Formation for Test Compounds

Compound	State	$\Delta G_f^{o\prime}$ (Kcal/mol)	Ref.
Benzene	aq	32.58	a*
Toluene	aq	29.56	c*
Phenol	aq	-11.18	d*
o-Xylene	ℓ	- 5.84	d
m-Xylene	ℓ	- 6.075	d
p-Xylene	ℓ	- 5.84	d
o-Nitrophenol	aq	- 1.55	e
m-Nitrophenol	aq	- 3.02	e
p-Nitrophenol	aq	- 2.47	e
o-Chlorophenol	aq	-11.55	e
m-Chlorophenol	aq	-11.55	e
p-Chlorophenol	aq	-11.55	e
Catechol	aq	-59.60	d*
Resorcinol	aq	-51.28	d*
Hydroquinone	aq	-49.39	d*
Acetate	aq	-88.29	b
HCO_3^-	aq	-140.26	b
NH_4^+	aq	-19.0	b
H^+	aq	- 9.67	b
$C\ell^-$	aq	-31.37	b
H_2O	aq	-56.69	b
CH_4	aq	- 8.22	b
CO_2	aq	-92.26	b

*Data from original source were modified for aqueous solution.

(a) Lange Handbook of Chemistry, 1979

(b) Handbook of Chemistry and Physics, 1987

(c) Parks and Huffman, 1932

(d) Karapetyants and Karapetyants, 1970

(e) Values calculated according to (c) from changes in free energies accompanying modifications of benzene

Table 2 Free Energies for Various Reactions of Test Compounds*

Compound	$\Delta G'^{\circ}$ (Kcal/mol) Homoacetic acid fermentation	Methanogenic fermentation
Benzene	13.68	- 32.79
Toluene	36.90	- 33.78
Phenol	0.75	- 37.65
o-Chlorophenol	- 39.92	- 70.26
m-Chlorophenol	- 39.92	- 70.26
p-Chlorophenol	- 39.92	- 70.26
o-Xylene	67.52	- 2.47
m-Xylene	67.76	- 2.23
p-Xylene	67.52	- 2.47
o-Nitrophenol	-106.73	-137.71
m-Nitrophenol	-105.26	-136.24
p-Nitrophenol	-105.81	-136.79
Catechol	- 7.52	- 37.84
Resorcinol	- 15.84	- 46.16
Hydroquinone	- 17.73	- 48.05

*Based on data in Table 1

Example:

Homoacetic acid fermentation of phenol: $C_6H_6O + 5H_2O \rightarrow 3H_3\ COO^- + 3H^+ + 2H_2$

Methanogenic fermentation of phenol: $C_6H_6O + 6.5H_2O \rightarrow 3.5CH_4 + 2.5HCO_3^- + 2.5H^+$

compounds were present in a 2 L, foil-covered glass reservoir at a level that was two times as concentrated as the feed concentration. A salt and vitamin nutrient solution was prepared in a separate 2 L, foil-covered glass reservoir at a level that was two times as concentrated as the actual feed concentration as indicated in Table 3. The substrate and the nutrient were pumped to the fermentor each at an average flow rate of 200 mL/day with two Master-flex, fixed-speed pumps (Cole-parmer 7543-02). A 30-day liquid retention time was maintained with these feed flow rates throughout this study. The fermentor was operated at a temperature of 35°C and the pH was maintained at 7.0 with sodium bicarbonate. Figure 6 shows the schematic diagram of the fermentor system.

b. Acetate-enriched methanogenic culture. An acetate-enriched methanogenic culture was developed from digested municipal wastewater sludge using a 20-L laboratory digester operated continuously at a 30-day liquid retention time. The sludge was collected from the West Hickman Sewage Treatment Plant, Lexington, Kentucky. The digester assembly was similar to that of the phenol-fed fermentor with three Master-flex, fixed-speed feed pumps and a wet test gas meter (Figure 7). The initial substrate was acetic acid which was present in a 2-L glass reservoir at 67.5 g/L and this substrate was replaced by a mixture of 67.5 g/L acetic acid and 10 g/L methanol in the later stages of this study. The substrate was pumped to the digester at an average flow rate of 140 mL/day. A nutrient solution having the composition shown in Table 3 was fed to the digester at an average flow rate of 140 mL/day. In addition, a solution containing 25 g/L sodium bicarbonate was pumped to the digester at 100 mL/day to maintain a neutral pH. The digester system shown in Figure 7 was housed in a 35°C constant temperature room.

Serum bottle technique. A serum bottle modification of Owen et al. (1979) was used to evaluate the anaerobic biodegradability and toxicity of test

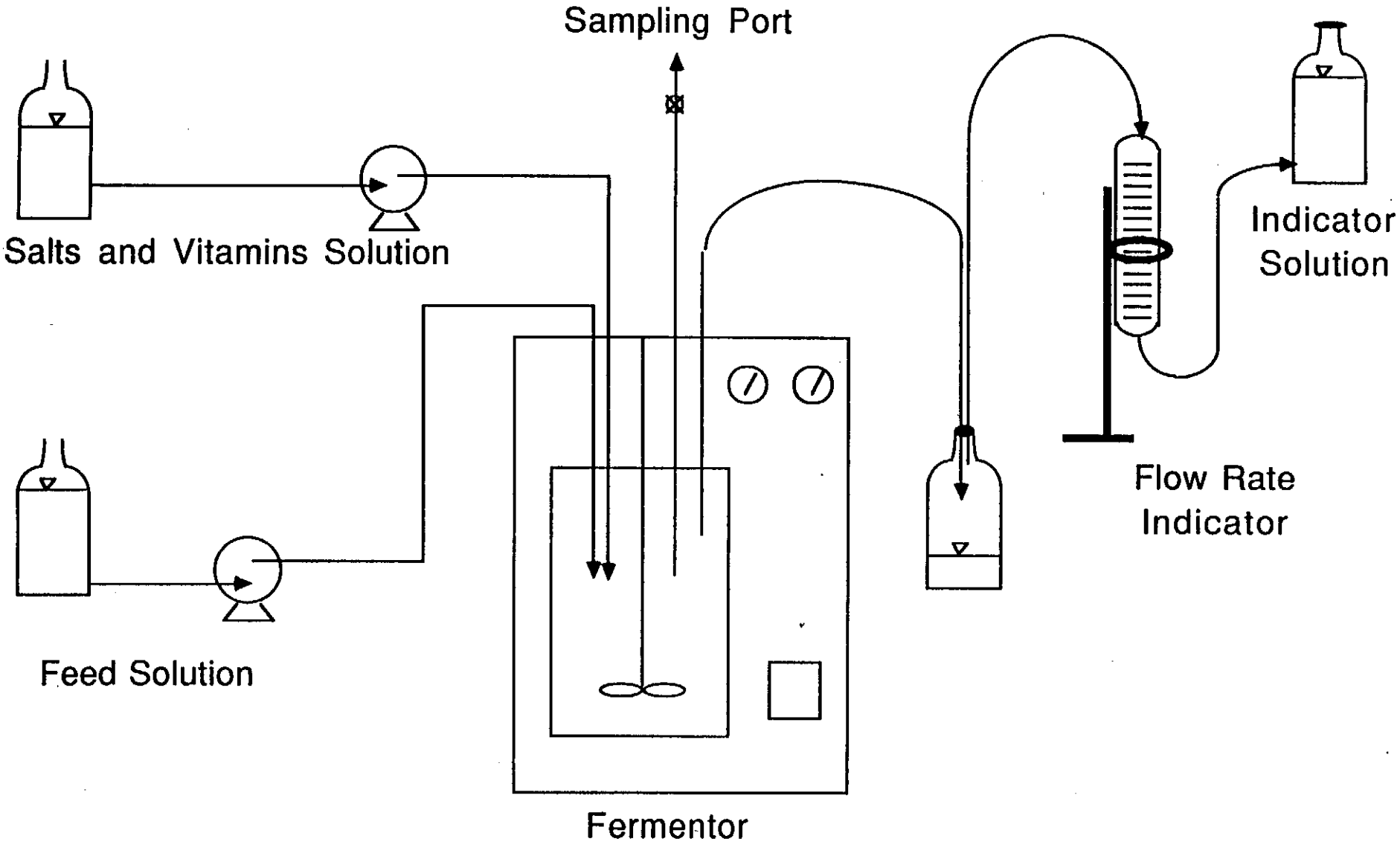

Figure 6. Schematic Diagram of the Phenol-enriched Fermentor

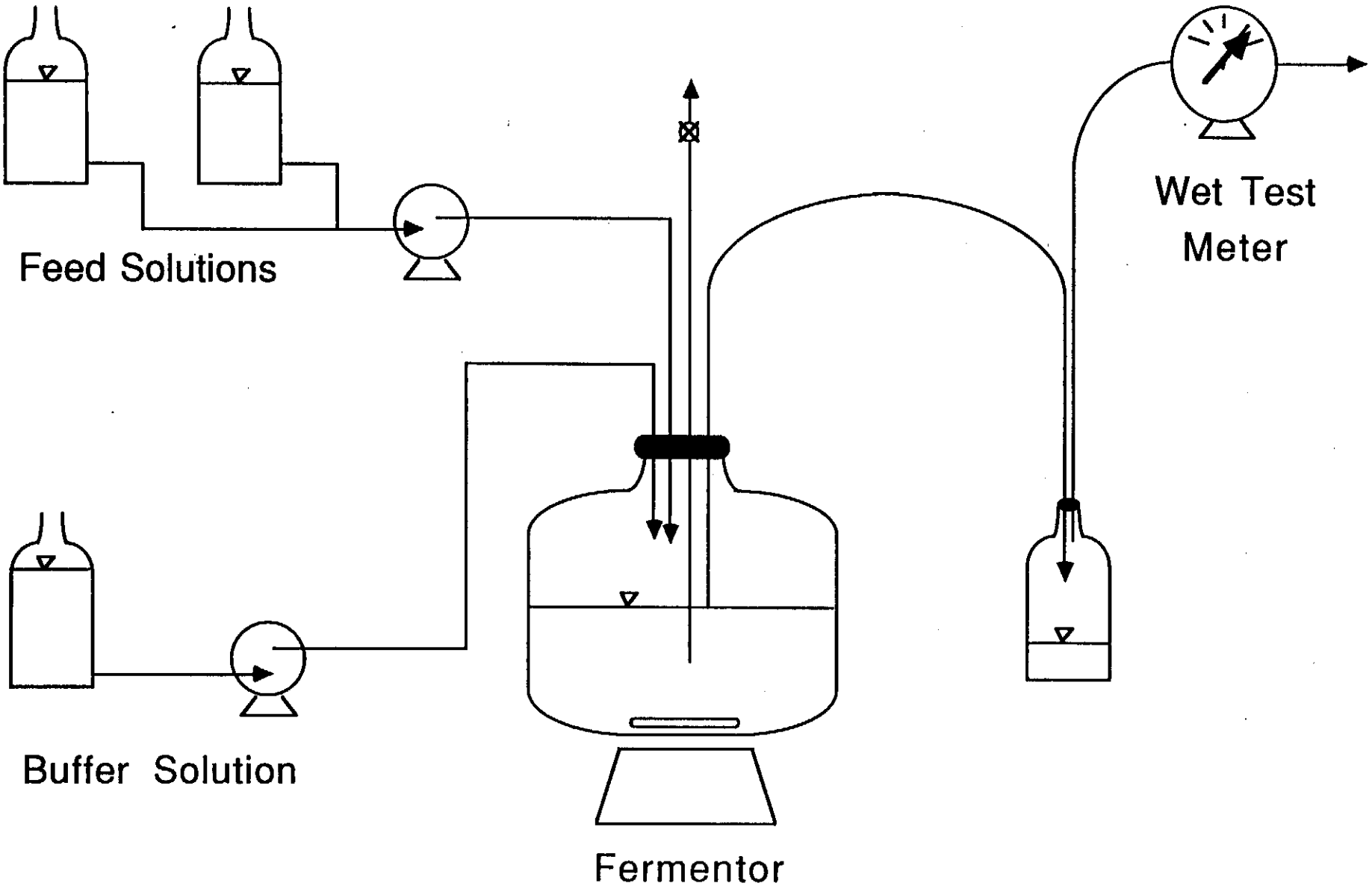

Figure 7. Schematic Diagram of the Acetate-enriched Fermentor

Table 3 Composition of Feed Salt and Vitamin

Compound	Concentration (mg/1)
$FeCl_3$	4.86
$MnCl_2 \cdot 2H_2O$	1.19
$ZnCl_2$	0.82
$CuCl_2 \cdot 2H_2O$	0.51
$CoCl_2 \cdot 6H_2O$	0.72
$Na_2B_4O_7.10H_2O$	0.29
$Na_3 \cdot$ Citrate	44.1
$(NH4)_6\ Mo_7O_{27} \cdot 4H_2O$	0.52
KH_2PO_4	102.08
$NaH_2PO_4 \cdot H_2O$	62.10
$(NH_4)_2SO_4$	39.60
NH_4cl	369
$Cacl_2 \cdot 2H_2O$	44.10
$Mgcl_2 \cdot 6H_2O$	60.98
$Nicl_2 \cdot 6H_2O$	0.50
Biotin	0.014
Folic acid	0.014
Pyridoxine hydrochloride	0.075
Riboflavin	0.038
Thiamin	0.038
Nicotinic acid	0.038
Pantothenic acid	0.038
B_{12}	0.00075
P-aminobenzoic acid	0.038
Thioctic acid	0.038

compounds. Serum bottles of 125 mL containing 100 mL growth media and seed inocula were incubated with test compounds at 35°C and the liquid and gas phases were sampled periodically by syringe extraction for subsequent analyses.

a. Screening test. A defined medium containing nutrients and vitamins needed for the growth of methanogenic cultures were prepared from a concentrated stock solution (Table 4) according to the procedure given in Table 5. The defined medium was transferred to serum bottles under anaerobic conditions as depicted in Figure 8. Serum bottles, gas tubing, and transfer glassware were initially gassed with an oxygen-free mixture of 30% CO_2 and 70% N_2. Oxygen in the gas mixture was removed by passing the mixture through a heated (450°C) silica glass tube filled with light copper turnings (Sargent-Welch Scientific Co., Skokie, Illinois). Serum bottles of 125 mL volume were gassed at a flow rate of approximately 0.5 L/min for 10 minutes with the same gas mixture prior to introduction of the culture medium. This medium was anaerobically pipetted by opening the serum bottles and medium flasks and purged with the gas mixture. After 80 mL volume of the prereduced medium was transferred to the respective serum bottles, 20 mL of seed inocula from the phenol-enriched culture were added to each bottle. Finally, the test compounds were added to these bottles at desired concentrations. The serum bottles were then sealed with butyl rubber stoppers while simultaneously removing the gas flushing needle. Resazurin was used as an indicator of reduced conditions. Sodium sulfide was added to 0.5 g/L to provide a reducing environment. Bottles were then shaken at 35°C. Duplicates were run for all test compounds. After equilibration for one hour at the incubation temperature, gas volumes were zeroed to ambient pressure with a syringe and the bottles were ready for incubation and sampling.

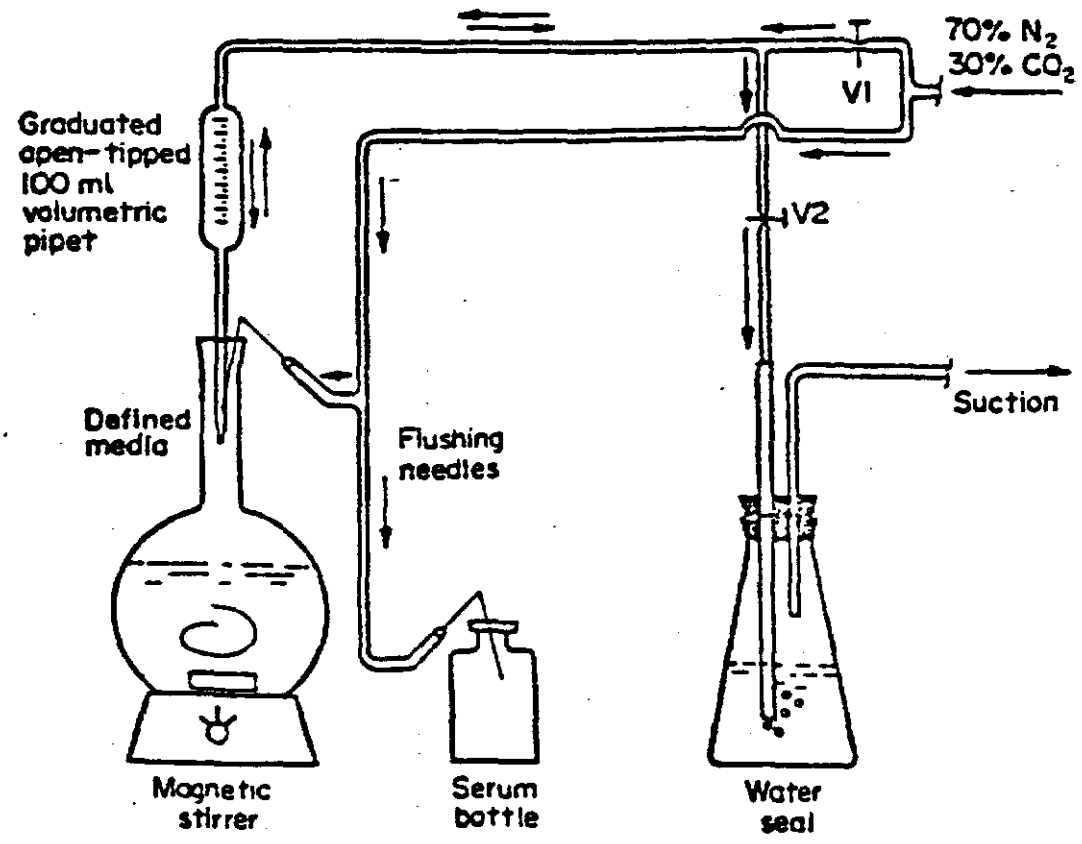

Figure 8. Anaerobic Transfer of Defined Media for Biodegradability (after Owen et al., 1979)

Table 4. Stock Solutions for Preparation of Defined Media

Solution	Compound	Concentration (g/L)
S_1	Resazurin	1
S_2	$(NH_4)_2HPO_4$	26.7
*S_3	$CaCl_2 \cdot 6H_2O$	16.7
	NH_4Cl	26.6
	$MgCl_2 \cdot 6H_2O$	120
	KCl	86.7
	$MnCl_2 \cdot 4H_2O$	1.33
	$CoCl_2 \cdot 6H_2O$	2
	H_3BO_3	0.88
	$CaCl_2 \cdot 2H_2O$	0.18
	$Na_2MoO_4 \cdot 2H_2O$	0.17
	$ZnCl_2$	0.14
	Nitrilotriacetic Acid	1.1
S_4	$FeCl_2 \cdot 4H_2O$	40
S_5	Biotin	0.002
	Folic Acid	0.002
	Pyridoxine Hydrochloride	0.01
	Riboflavin	0.005
	Thiamin	0.005
	Nicotinic Acid	0.005
	Pantothenic Acid	0.005
	B_{12}	0.0001
	p-Aminobenzoic Acid	0.005
	Thioctic Acid	0.005

* pH adjusted to 7.0 with KOH.

Table 5. Preparation of Defined Media

Step	Procedure
1.	Add 1 liter of deionized water to 2 liter volumetric flask
2.	Add the following: 1.8 mL S_1 5.4 mL S_2 27 mL S_3
3.	Add deionized water up to 1,800 mL mark
4.	Boil for 15 min while flushing with N_2 gas at approximately 1 L/min
5.	Add 1.0 g L-cysteinehydrochloride·H_2O
6.	Cool to room temperature (continue flushing with N_2)
7.	Add the following: 18 mL S_5 1.8 mL S_4 1.0 g $Na_2S·9H_2O$
8.	Change gas to 30% CO_2, 70% N_2 mixture and continue flushing at 1 L/min
9.	Add 7.2 g $NaHCO_3$ as powder
10.	Bubble 30% CO_2 : 70% N_2 gas mixture through porous diffuser until media pH stabilizes at approximately 7.0
11.	Carefully seal volumetric flash while minimizing introduction of air into container

b. Biodegradation test. The procedure for preparing the biodegradation test was the same as the screening test except that no defined medium was transferred to serum bottles. A 100-mL volume of culture was transferred directly from the phenol-fed fermentor to each 125-mL serum bottle while under the purge of an oxygen-free, 30% CO_2 and 70% N_2 gas mixture.

c. Toxicity test. In the toxicity test, assay bottles were prepared similar to the biodegradability test with test compounds, seed inocula, and reducing agents. In addition, a spike containing 200 mg/L phenol or 500 mg/L acetic acid was added to each bottle as the substrate to determine the effect of test compounds on phenol degradation and acetate utilization. Acetate was selected because it is the major precusor of methane and it accounts for approximately 70% of the methane produced from complex organic materials (McCarty, 1964; Jeris and McCarty, 1965; Smith and Mah, 1966). Phenol was selected as the substrate to evaluate the inhibitory effects of test compounds on aromatic ring-degrading bacteria. Duplicates were run for all samples, including the seed blanks and controls. The control bottles were prepared as test bottles while no test compounds were added. The phenol control received only 200 mg/L phenol and the acetate control received only 500 mg/L acetic acid.

Analytical Methods

Aqueous and gas samples were withdrawn from test bottles with syringes at proper intervals for the following analyses:

Gas analysis. Gas samples were analyzed for methane, carbon dioxide, nitrogen, and oxygen using a Fisher Model 1200 gas partitioner. Helium was used as the carrier gas while certified calibration standards was used to calibrate the gas analyzer. Gas production was measured by displacement of the plunger in an appropriate size wetted glass syringe.

Analysis for phenols. The analysis for phenolic compounds was conducted on

filtered samples by a Varian Model 5020 liquid chromatograph consisting of three microprocessor-controlled pumps and a model UV-100 variable wavelength UV detector. Detection wavelength was set at 280 nm. Compounds were separated by using a MicroPak MCH-5 C_{18} reverse phase column at ambient temperature. The mobile phase consisted of water (buffered at pH 2.54 with 0.005 M KH_2PO_4), methanol (50:50) with a flow rate of 1 mL/min. For samples spiked with only one test compound, a 30% water : 70 % acetonitrile mobile phase was used.

Volatile Organic Acids Analysis. Samples for volatile fatty acids analyses were acidified to pH 2 and filtered (0.45 μm) prior to gas chromatographic separation using a Varian Model 3400 gas chromatograph and a 92cm long, 2mm I.D. glass column packed with 0.3% carbowax with 0.1% phosphoric acid on 80/100 carbopack.

Biomass Determination. Biomass concentration was determined by measuring volatile suspended solids (Standard Methods, 1985) as well as protein content. The protein content was determined by the modified method of Lowry et al. (Markwell et al., 1978).

III. RESULTS AND DISCUSSION

Screening Test

The anaerobic biodegradability of the fourteen test compounds was first screened using the phenol-enriched methanogenic culture as the seed inocula. Table 6 summarizes total gas production in test bottles and seed-blank bottles. Phenolic compounds were directly spiked into test bottles at desired concentrations while water-insoluble benzene, toluene, and xylenes were first dissolved in 100 uL ethanol and then added to test bottles. The data in Table 6 indicate that total gas production in bottles containing benzene, toluene, and xylenes did not exceed those in seed-blank bottles after incubated for two to six months. The seed-blank bottles of the water-insoluble compounds were spiked with 100 uL ethanol and produced very high levels of background gas. Compared with such background gas production, none of the test bottles containing water insoluble aromatics produced significantly more gas than seed blanks. Therefore, the gas data indicate that these compounds were not degraded to methane in contrast to thermodynamic reasoning shown in Table 2. In addition, each of these compounds at a concentration of 800 mg/L exhibited inhibition as indicated by the decreased gas production.

Phenolic compounds have higher solubilities and are more biodegradable than the water-insoluble aromatics. Net gas production was observed with 50 - 500 mg/L catechol, 100 - 700 mg/L hydroquinone, and 50 - 100 mg/L resorcinol. Inhibition was noted at higher concentrations as evidenced by reduced gas production. Net gas production was also observed with 10 - 50 mg/L 4-NP. The nitrophenols and the chlorophenols seemed to be more inhibitory than the hydroxyphenols. Between 100 and 500 mg/L, the nitrophenols and the chlorophenols reduced gas production. However, the exact biodegradable range was not clearly indicated by the gas data. Furthermore, biodegradation of

Table 6 Summary of Screening Test with the Phenol-enriched Culture

Nominal Concentration (mg/ℓ)	Total gas production, mℓ													
	2-NP	3-NP	4-NP	Catechol	Hydroquinone	Resorcinol	2-CP	3-CP	4-CP	Toluene	Benzene	o-Xylene	m-Xylene	p-Xylene
0 (seed blank)	3.2	5.7	2.8	9.0	9.0	9.7	13.5	13	10	87*	77*	91*	80*	93*
10	1.6	4.5	7.5	10.5	3.5	9.6	5.0	10.5	9.0	88	87	82	72	74
50	3.4	5.0	10.5	14	9.5	17	8.0	13.4	8.0	87	91	81	80	77
100	1.8	2.0	0	18	17	14	8.0	4.1	13	81	88	85	85	77
200	0	0	0.6	25.5	27	9	9.5	2.0	8.8	77	81	75	82	77
300	0	0	1.0	40	37.5	5	9.5	6.0	4.0	—	—	—	—	—
400	–	–	–	—	—	–	–	–	–	—	88	69	80	86
500	0	0	0	57.5	55	9.5	2.0	0.5	5.5	—	—	—	—	—
700	0	0	0	10.5	60	8	2.0	1.0	2.0	—	—	—	—	—
800	–	–	–	—	—	–	–	–	–	—	—	7.0	67	65
1000	0	0	0	14	27.5	6.0	2.5	1.0	3.0	—	—	—	—	—
Length of Incubation (month)	7.8	7.7	7.5	7.5	7.3	7.3	7.2	7.0	7.0	6.0	5.8	2.5	5.5	5.3

*seed blank spiked with 100 μℓ ethanol

nitrophenols and chlorophenols were not conclusive due to significant variation in seed-blank gas data.

Biodegradation Test

The anaerobic biodegradability of nine phenolic compounds was further examined with the phenol-enriched methanogenic culture. Table 7 lists the times required for complete disappearance of phenolic compounds at various concentrations in the phenol-enriched culture. Chlorophenols were the most refractory among the three substituent groups. After a long incubation period of more than eight months, complete disappearance of chlorophenols was observed only with the lowest concentration tested (5 mg/L). The relative rates of degradation were in the order of ortho meta para. A similar pattern of degradation of chlorophenols has also been found in sewage sludges (Boyd and Shelton, 1984; Hrudey et al., 1987), with 4-chlorophenol requiring acclimation periods between 16 and 20 weeks before degradation occurred. The long lag period and low biodegradable concentrations noted in this study indicate that a culture acclimated to phenol did not utilize chlorophenols.

The nitrophenols were degraded in the phenol-enriched culture at higher rates than the chlorophenols. All three nitrophenol isomers at concentrations as high to 100 mg/L disappeared within an incubation period of two months. However, complete disappearance of 200 mg/L 4-NP was observed within three months. Similar to chlorophenols, nitrophenols were degraded preferentially in the order of ortho > meta > para. At the end of incubation, excess methane was observed only with lower initial concentrations (30 mg/L and lower), indicating conversion of nitrophenols to methane at these concentrations (Table 8). Due to their strong inhibition, methane production from higher concentrations of nitrophenols (50 mg/L and higher) was less than that from seed blanks. The reduction of nitro groups to

Table 7 Biodegradability of Phenolic Compounds in Phenol-enriched Methanogenic Culture

Nominal Concentration (mg/ℓ)	Time needed for complete degradation, hour									
	Phenol	Catechol	Resorcinol	Hydroquinone	2-CP	3-CP	4-CP	2-NP	3-NP	4-NP
5	—	—	—	—	5760	5910	6012	—	—	—
10	5	7	1126	1054	N	N	N	343	343	406
20	—	—	—	—	N	N	N	—	—	—
30	7	17.5	1126	1054	—	—	—	343	343	406
40	—	—	—	—	N	N	N	—	—	—
50	10	36	5318	1054				343	657	478
100	24	59	5318	1200				512	983	1538
200	39	126	N	5900						1842
400	91	307		5900						
800	176	1076		5900						
1000	254	N		5900						
2000	N*									

*Not degraded

Table 8 Methane Production from Nitrophenols

Compound	Concentration, mg/ℓ	CH_4 Production at STP, mℓ
2-NP	0 (seed blank)	8.0
	10	7.7
	30	9.2
	50	6.3
	100	6.3
3-NP	0 (seed blank)	9.4
	10	10.9
	30	10.7
	50	8.4
	100	3.0
4-NP	0 (seed blank)	8.5
	10	10.3
	30	11.9
	50	3.2
	100	3.2
	200	3.1

amino groups in the aromatic rings in anaerobic environments is well established and probably occurred in the phenol-enriched culture since an unidentified product was noted.

The nitrophenols were less persistent than the chlorophenols in the phenol-enriched culture as was previously found in sewage sludges by Boyd et al (1983). However, acclimation of the phenol culture to nitrophenols required more than 300 hours which is considerably longer than the one week lag period observed previously with sewage sludges (Boyed et al., 1983). Thus, the phenol-enriched culture did not offer an advantage over digested sewage sludge in degrading nitrophenols.

Although complete disappearance of both chlorophenols and nitrophenols was noted for lower concentrations, the time course of degradation was not observed. The metabolic pattern of these compounds was typically a prolonged lag period followed by rapid degradation. Similar patterns were observed with hydroquinone and resorcinol. Resorcinol was completely degraded at 100 mg/L but not at 200 mg/L after more than 5000 hours incubation whereas complete degradation of 1000 mg/L hydroquinone was observed after nearly 6000 hours incubation. Young and Rivera (1985), however, noted that complete metabolism of approximately 400 mg/l hydroquinone in unacclimated digester inoculum took about 75 days which was shortened to about 28 days after the second spike. As compared to the previous results, the phenol-enriched culture did not degrade hydroquinone faster than the unacclimated digester sludge.

Phenol and catechol were degraded to methane over a wide range of concentrations. Figures 9-12 show their degradation over time at various initial concentrations. Corresponding methane production was shown in Figures 13 and 14 for phenol and catechol, respectively. The phenol-enriched culture was able to degrade phenol at concentrations as high to 1000 mg/L without apparent lag periods. Conversion of 1000 mg/L phenol was complete within 250

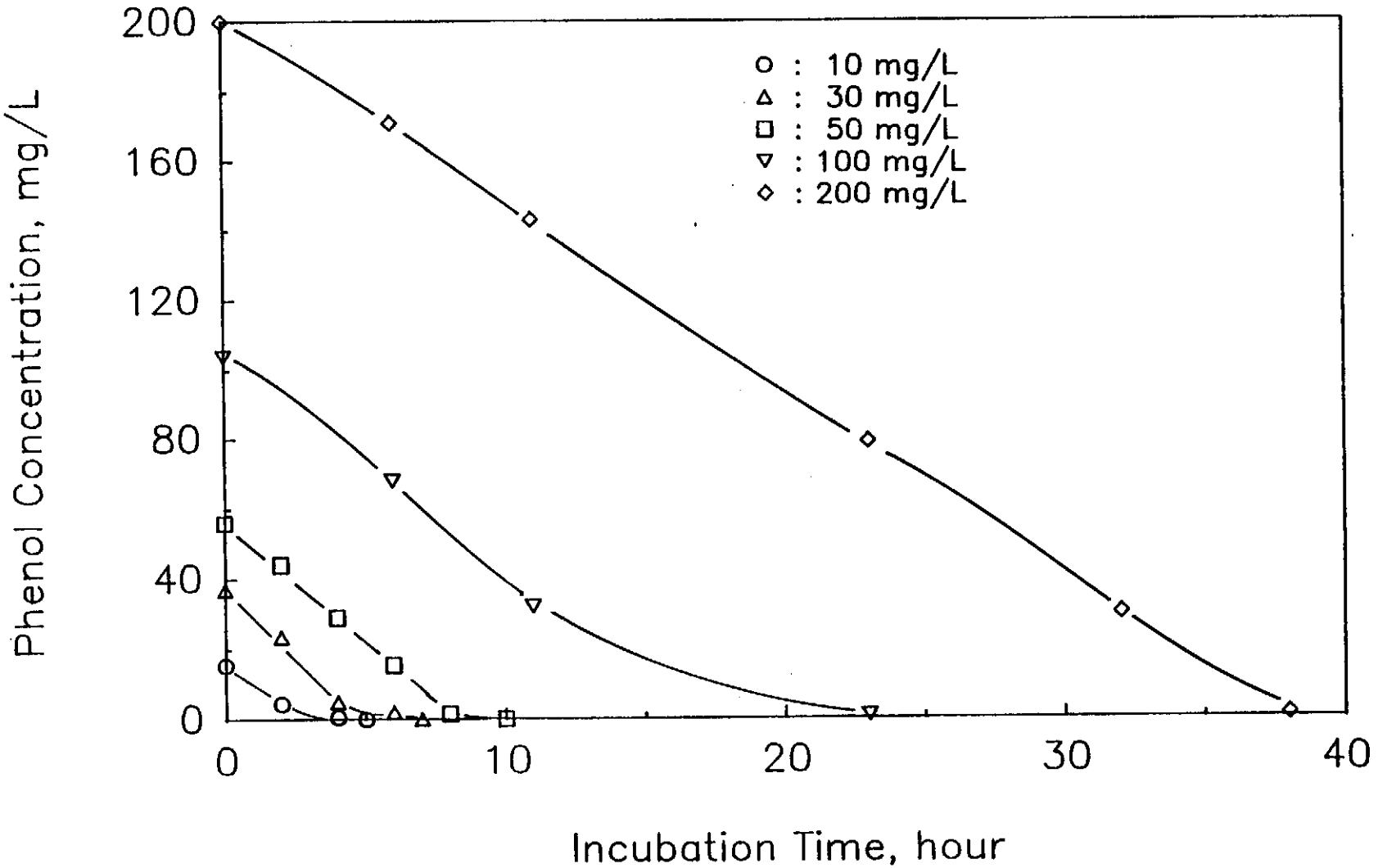

Figure 9. Biodegradation of Phenol in Phenol-enriched Culture: Lower Initial Concentrations

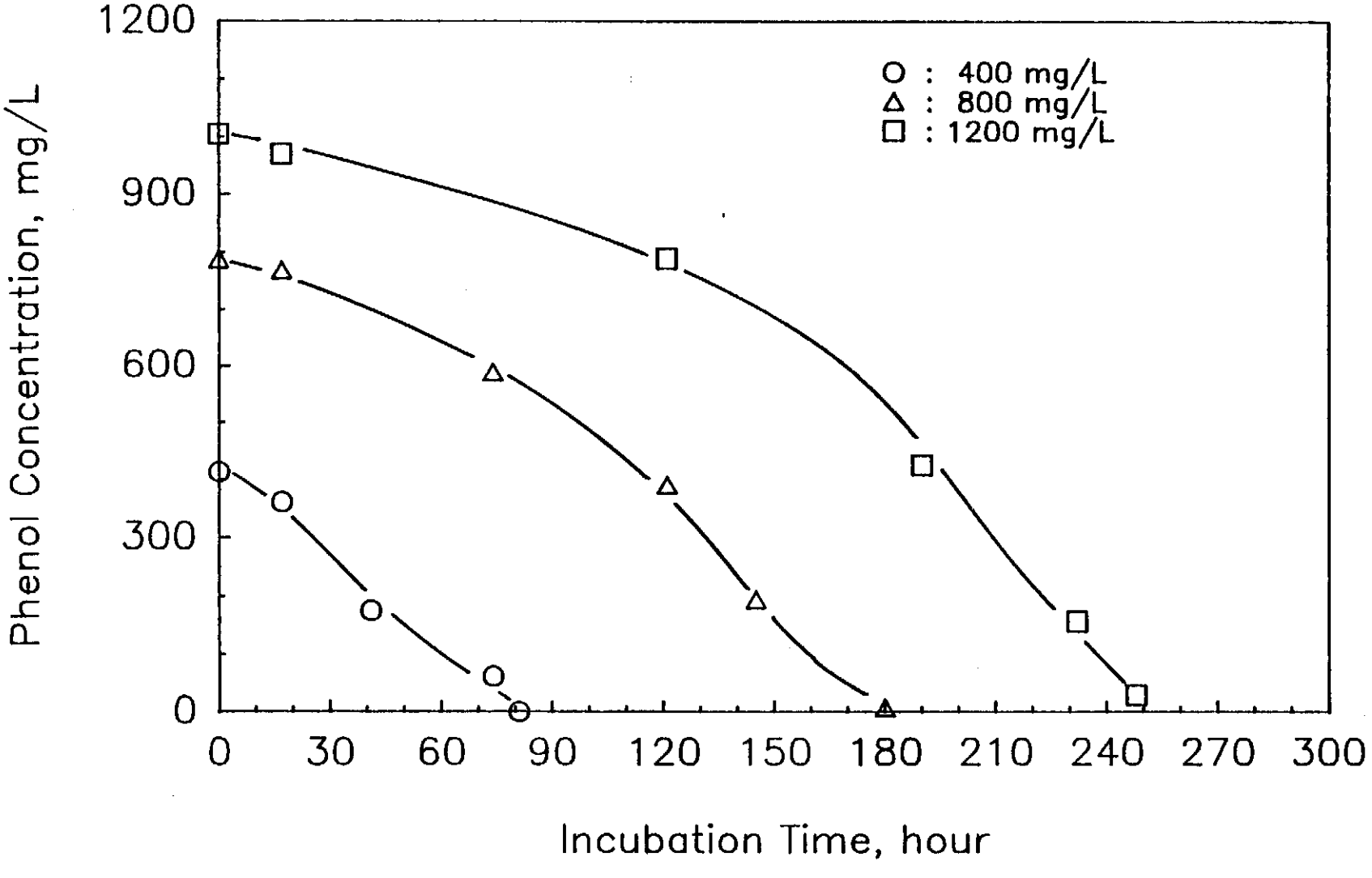

Figure 10. Biodegradation of Phenol in Phenol-enriched Culture: Higher Initial Concentrations

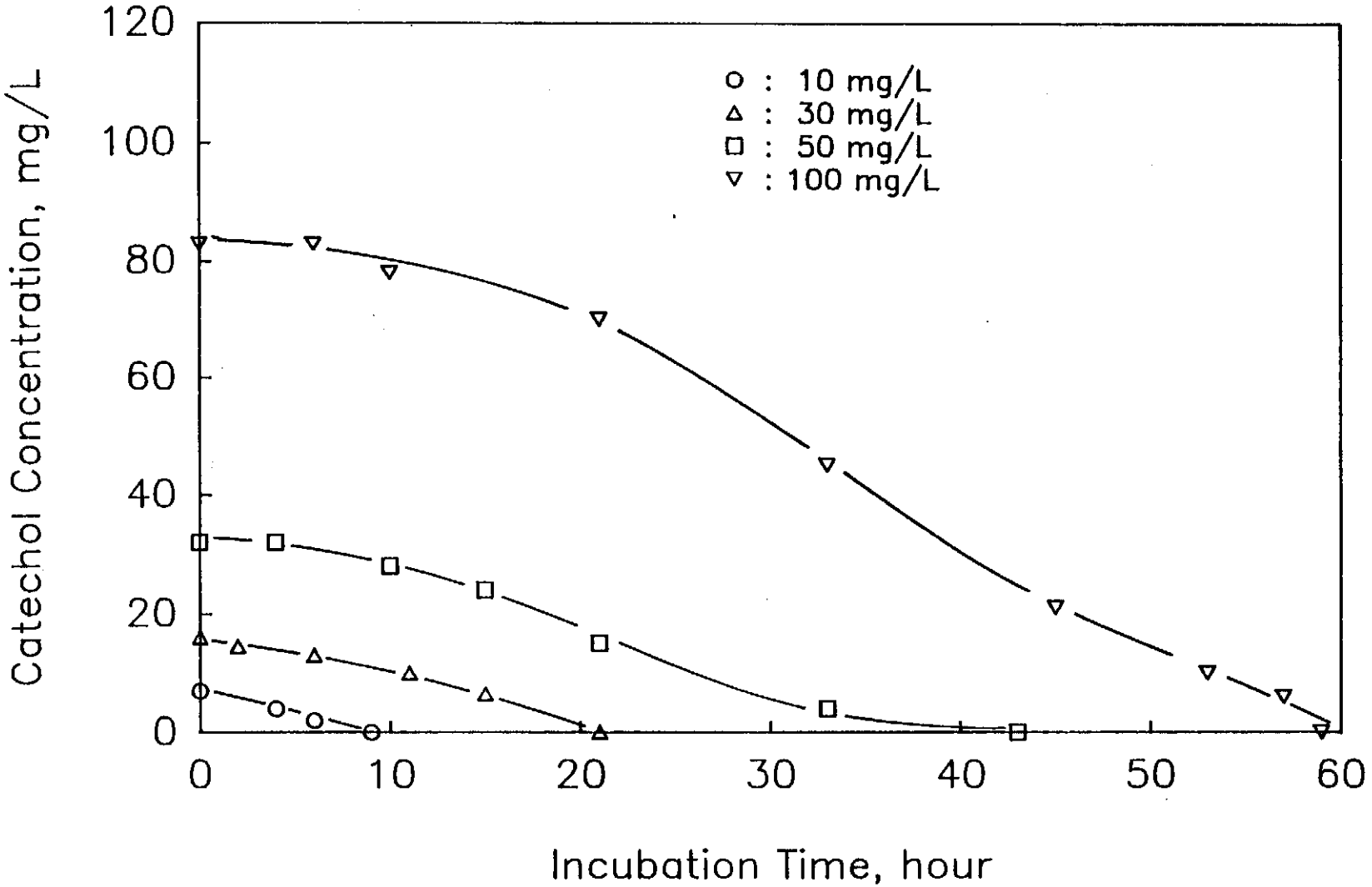

Figure 11. Biodegradation of Catechol in Phenol-enriched Culture: Lower Initial Concentrations

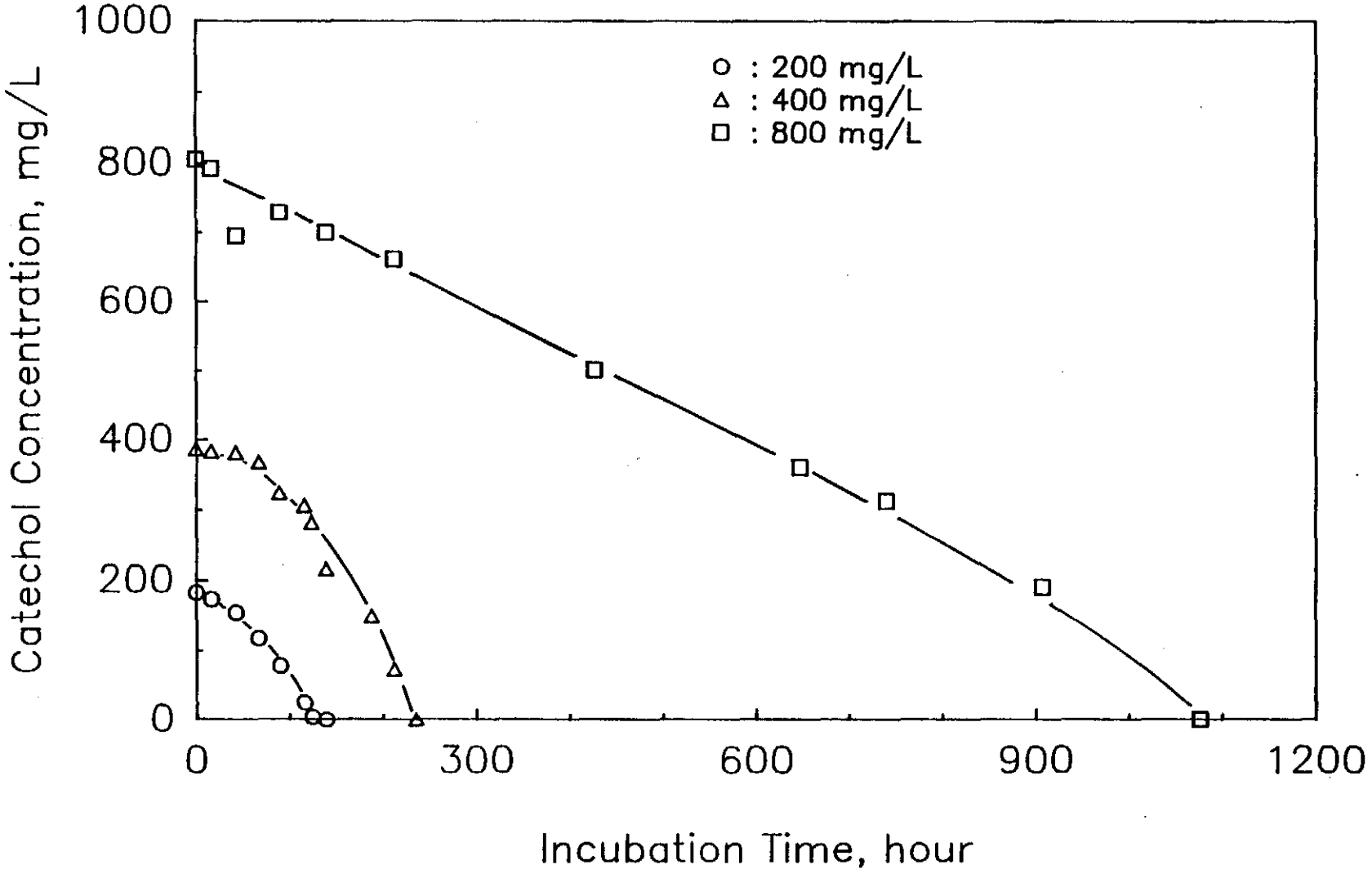

Figure 12. Biodegradation of Catechol in Phenol-enriched Culture: Higher Initial Concentrations

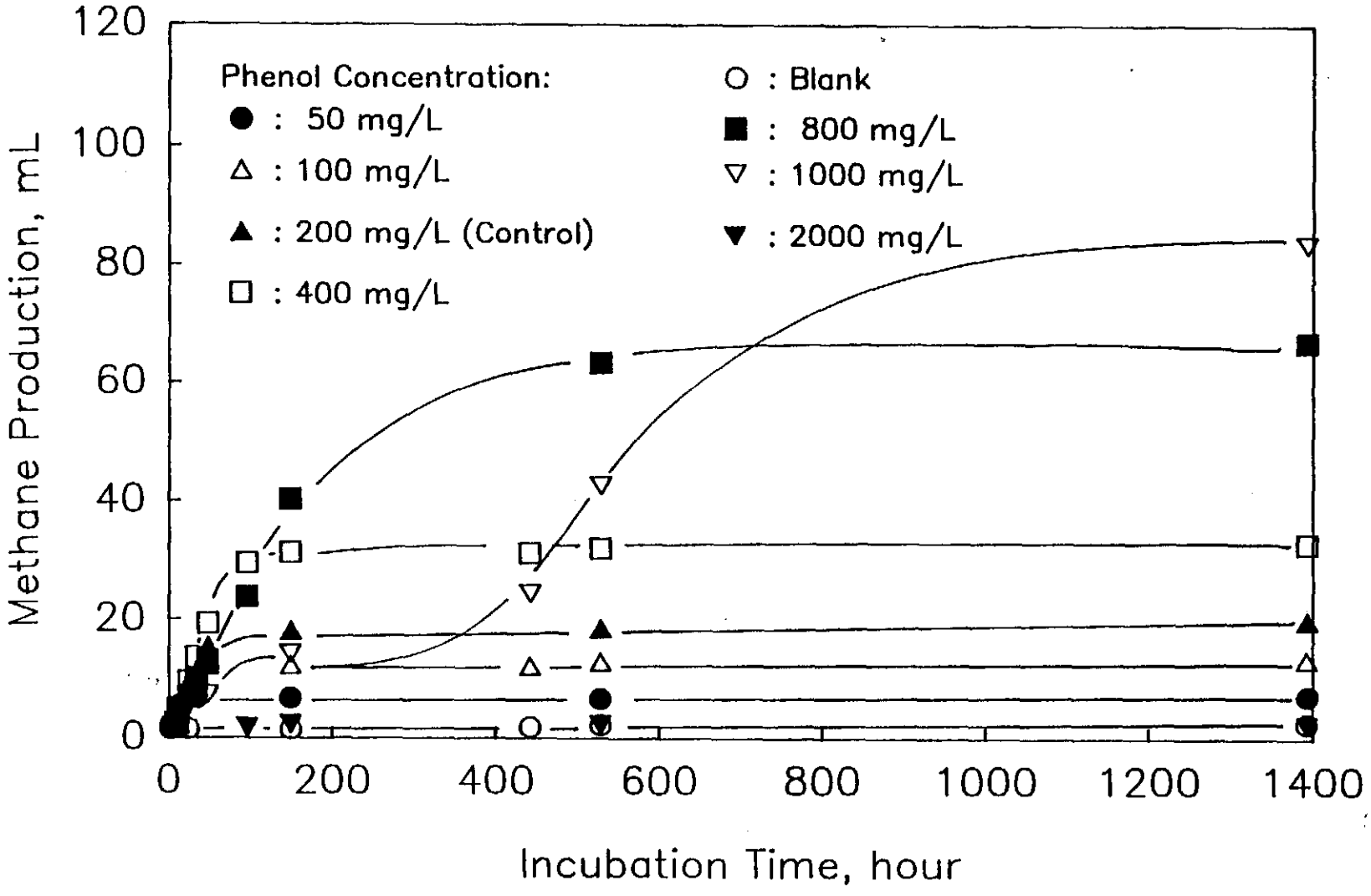

Figure 13. Methane Production from Phenol

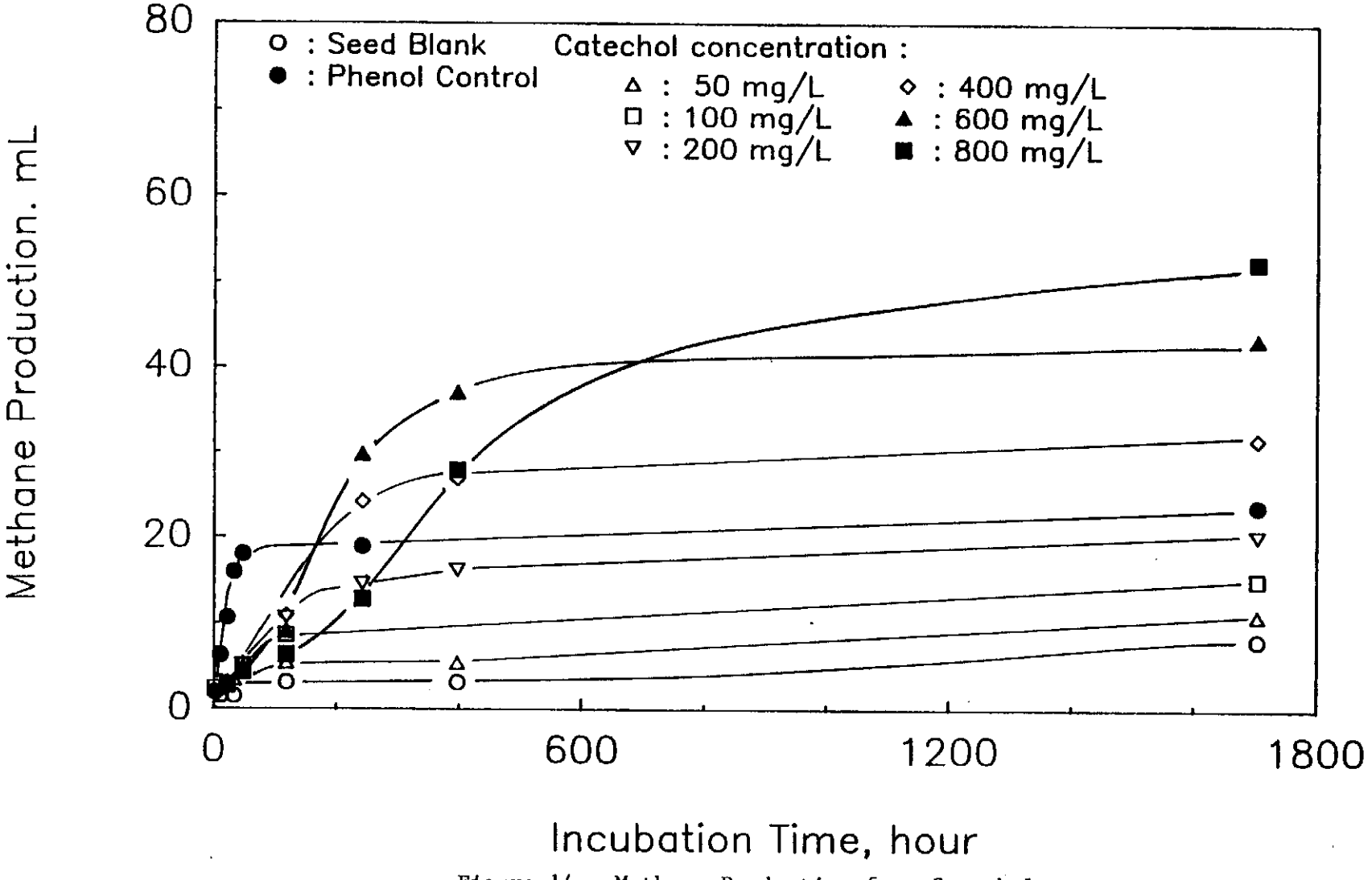

Figure 14. Methane Production from Catechol

hours. However, phenol at 2000 mg/L was not degraded even after two months incubation. For initial phenol concentrations of 800 and 1000 mg/L, the rates of phenol degradation increased with time. However, the growth rate of the bacterial population as determined with protein analyses was negligible throughout the incubation (Figures 15 and 16). Therefore, the increase in degradation rate may be due to an increase in biological activity instead of biomass growth. The rates of phenol degradation were rather constant at 200 mg/L and at lower initial concentrations while substrate inhibition occurred at 400 mg/L. The inhibitory effects of phenol at 1000 mg/L was severe and the activity of the phenol culture was completely ceased at 2000 mg/L. Similar results were reported by Wang et al (1989) in an earlier study using a phenol-enriched culture. Phenol at 1000 mg/L was not degraded in previous studies using unacclimated digester sludges (Fedorak and Hrudy, 1984; Blum et al. 1986). Therefore, degradation of higher concentrations of phenol can be accomplished at higher rates using phenol-enriched cultures.

Catechol was converted to methane at a lower rate than phenol. For example, complete degradation of 800 mg/L catechol required about 1100 hours while less than 200 hours was needed for 800 mg/L phenol. Substrate inhibition became severe at 800 mg/L and no biological activity was observed with 1000 mg/L catechol. Similar to phenol, biomass growth during catechol utilization was negligible with slight increase observed only after a very long incubation period (Figures 17 and 18). A lag period about 100 hours was noted before degradation of 400 mg/L catechol occurred. Healy and Young (1978) reported that utilization of 300 mg/L catechol by a methane digester effluent required an exceedingly long acclimation period of 4.5 weeks. Although the lag period was shortened to about one week during their second spike of catechol, much shorter lag was observed in this study with the

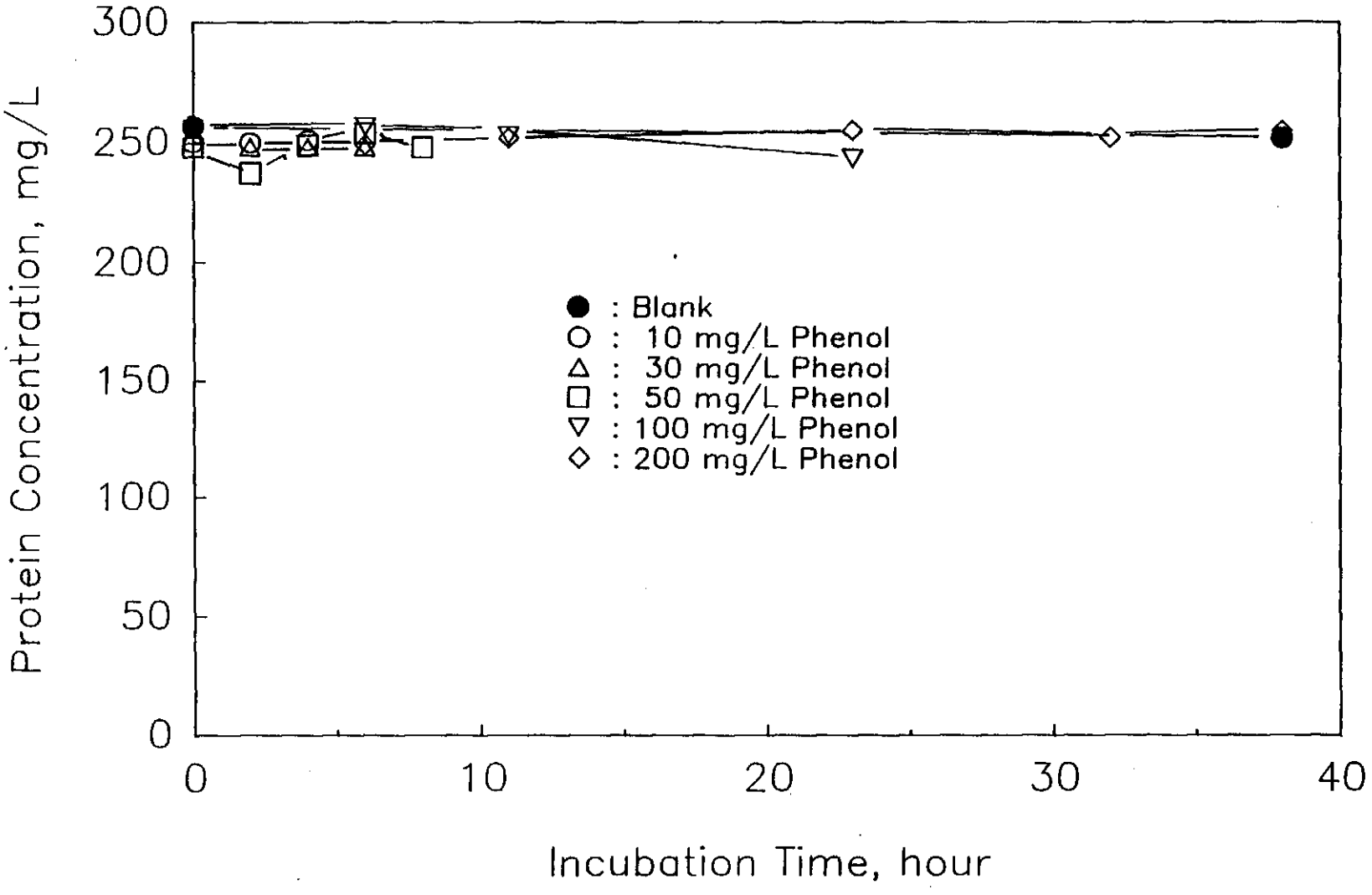

Figure 15. Growth of Biomass During Phenol Degradation: Early Stages

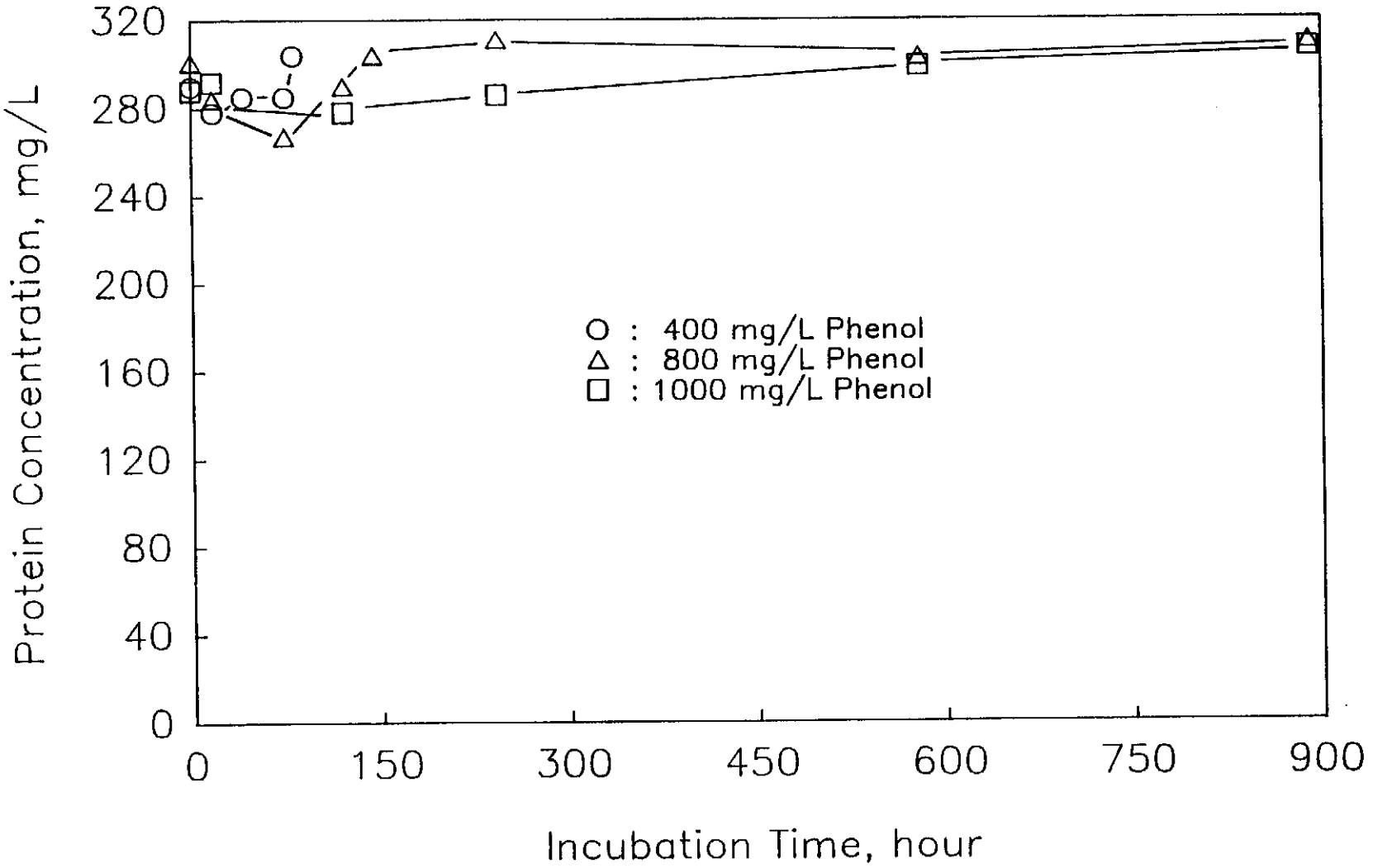

Figure 16. Growth of Biomass During Phenol Degradation: Entire Incubation

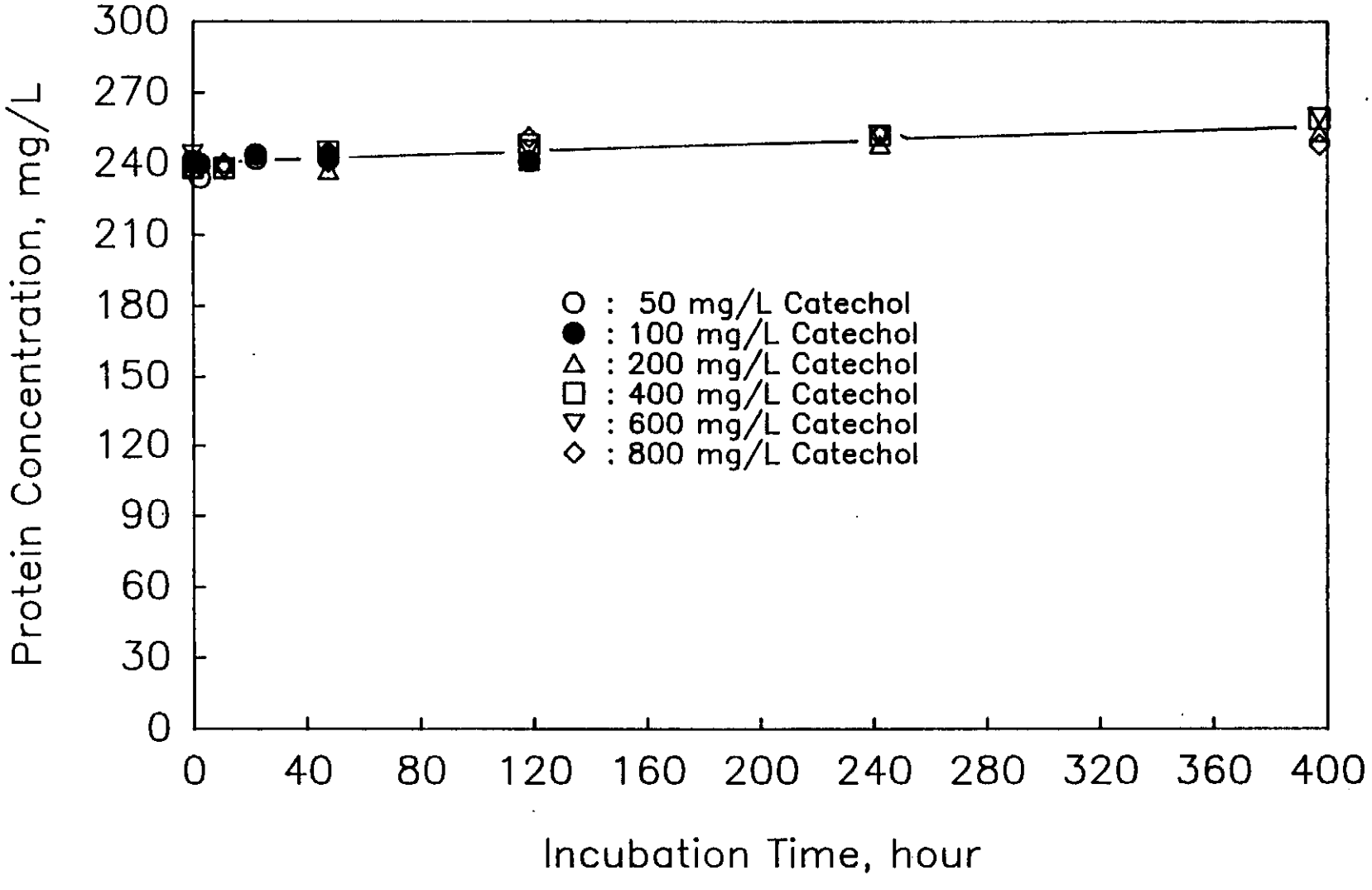

Figure 17. Growth of Biomass During Catechol Degradation: Early Stages

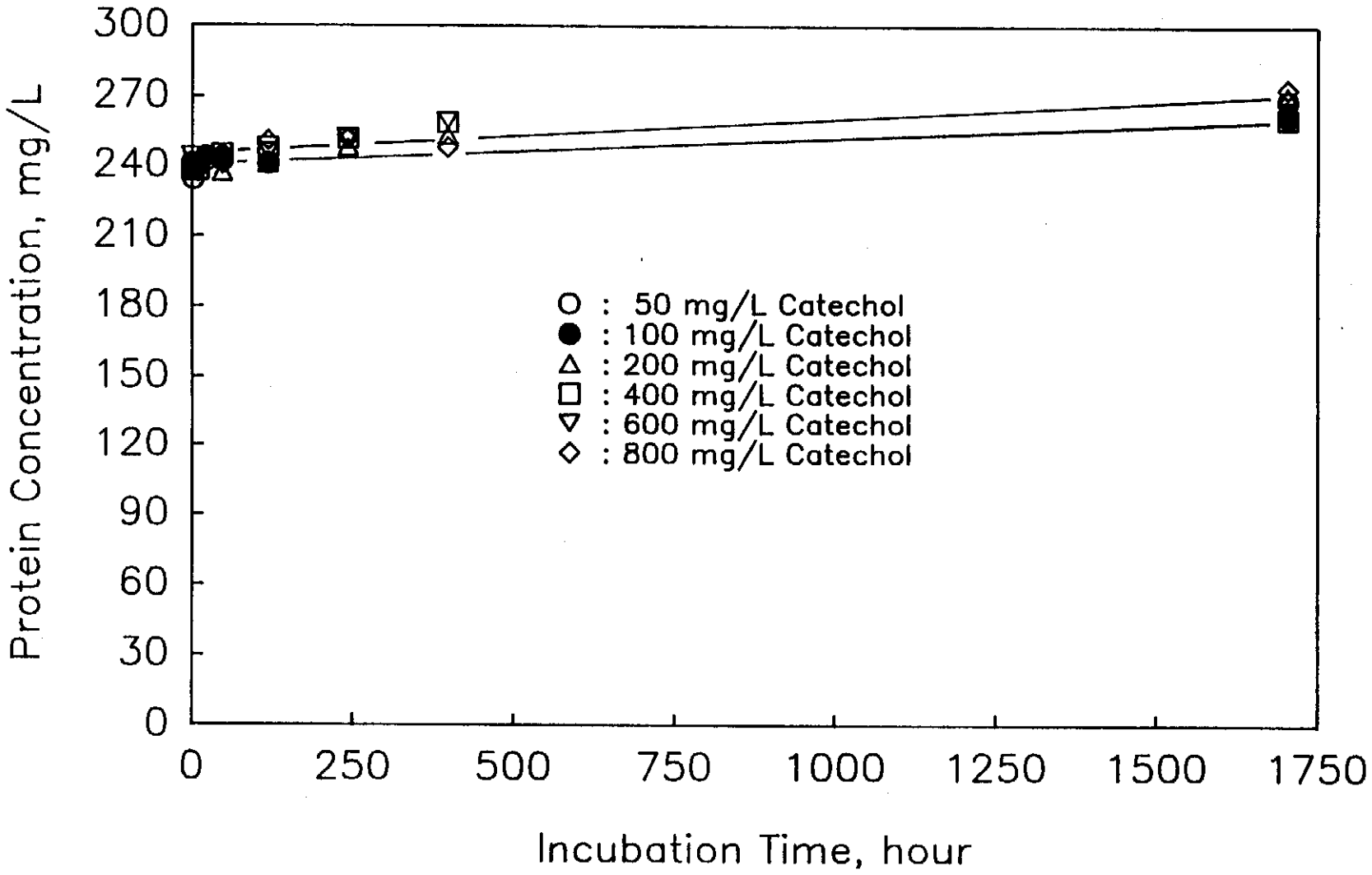

Figure 18. Growth of Biomass During Catechol Degradation: Entire Incubation

phenol-enriched methanogenic culture. The shorter lag period noted in this study suggested that the phenol-enriched culture was acclimated to catechol prior to batch degradation studies.

Inhibition of Acetate Utilization

The effect of concentration of phenolic compounds on acetate-utilizing methanogens was investigated with the acetate-enriched culture. Figures 19 - 35 show the cumulative methane production from the acetate-enriched culture in the presence of phenolic compounds at various concentrations. The methanogenic activity that remained after the addition of a phenolic compound was quantified as a percentage of the total methane production from the uninhibited control at the end of each incubation and is shown in Figure 36. Table 9 summaries the toxicity levels and concentrations observed for 50% reduction in methanogenic activity. Methane production from added acetate virtually ceased in the presence of 4000 mg/L phenol, indicating toxicity at this concentration. This finding was in agreement with earlier reports that toxicity level of phenol toward methanogens ranged between 3000 and 4000 mg/L (Fedorak and Hrudey, 1984; Blum et al. 1986). In comparison with the phenol data, the presence of chloro or nitro groups rendered phenol more inhibitory while hydroxyl group reduced inhibition. These findings are in agreement with previous results obtained with sewage sludges and lake sediments (Battersby and Wilson, 1989; Horowitz, 1982). Furthermore, the inhibitory effects of the three isomers of chlorophenol and nitrophenol did not vary significantly with the isomer similar to those found with cresols and ethylphenols using a phenol-enriched culture (Wang, et al.1988).

The data in Figure 36 also show that low levels of phenol and hydroquinone (200 mg/L and lower) enhanced methanogenic activity. Liquid phase analyses conducted at the beginning and the end of the incubation showed that both phenol and hydroquinone were degraded at concentrations as high to

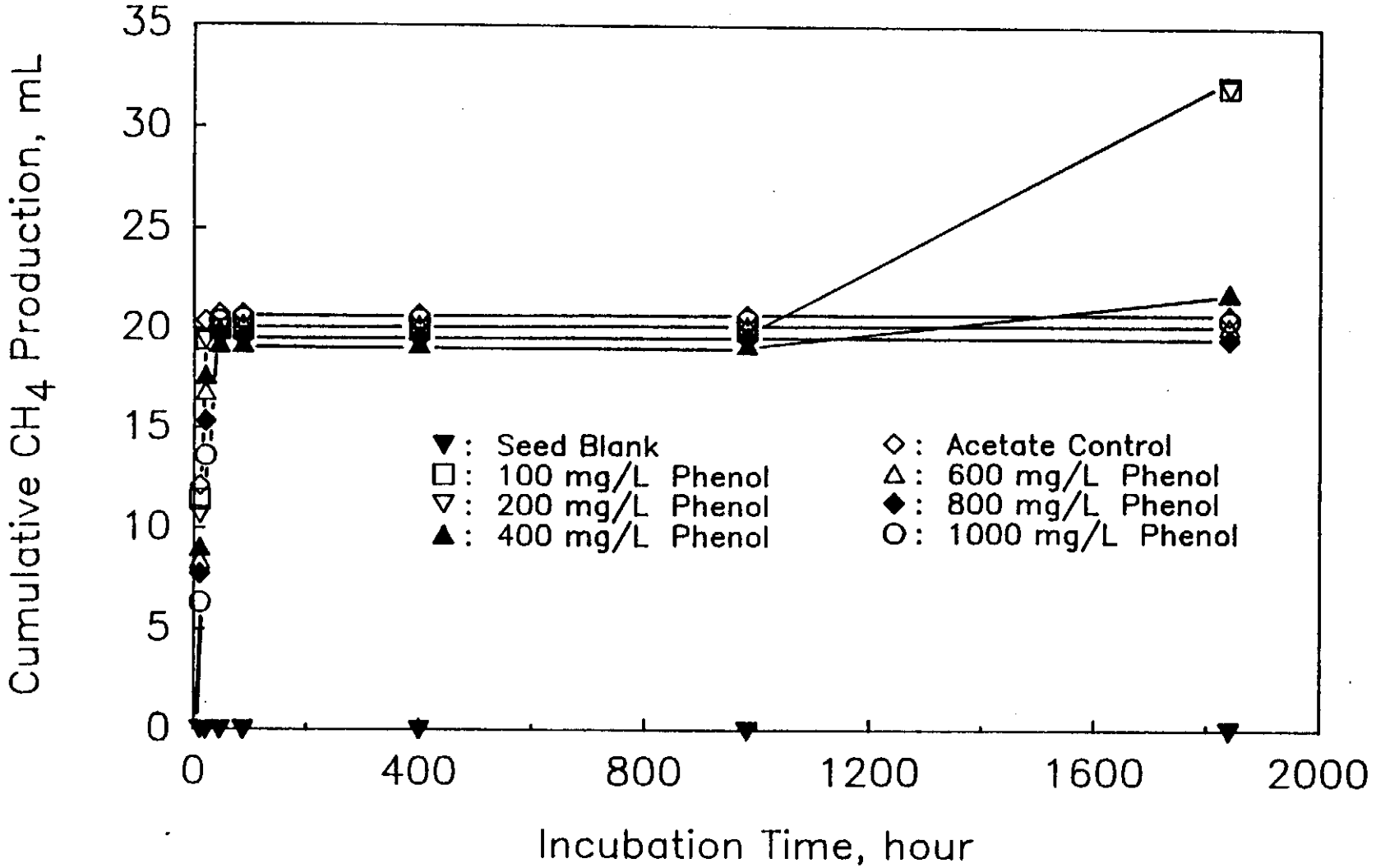

Figure 19. Methane Production from Added Acetate in the Presence of Phenol: Lower Concentrations

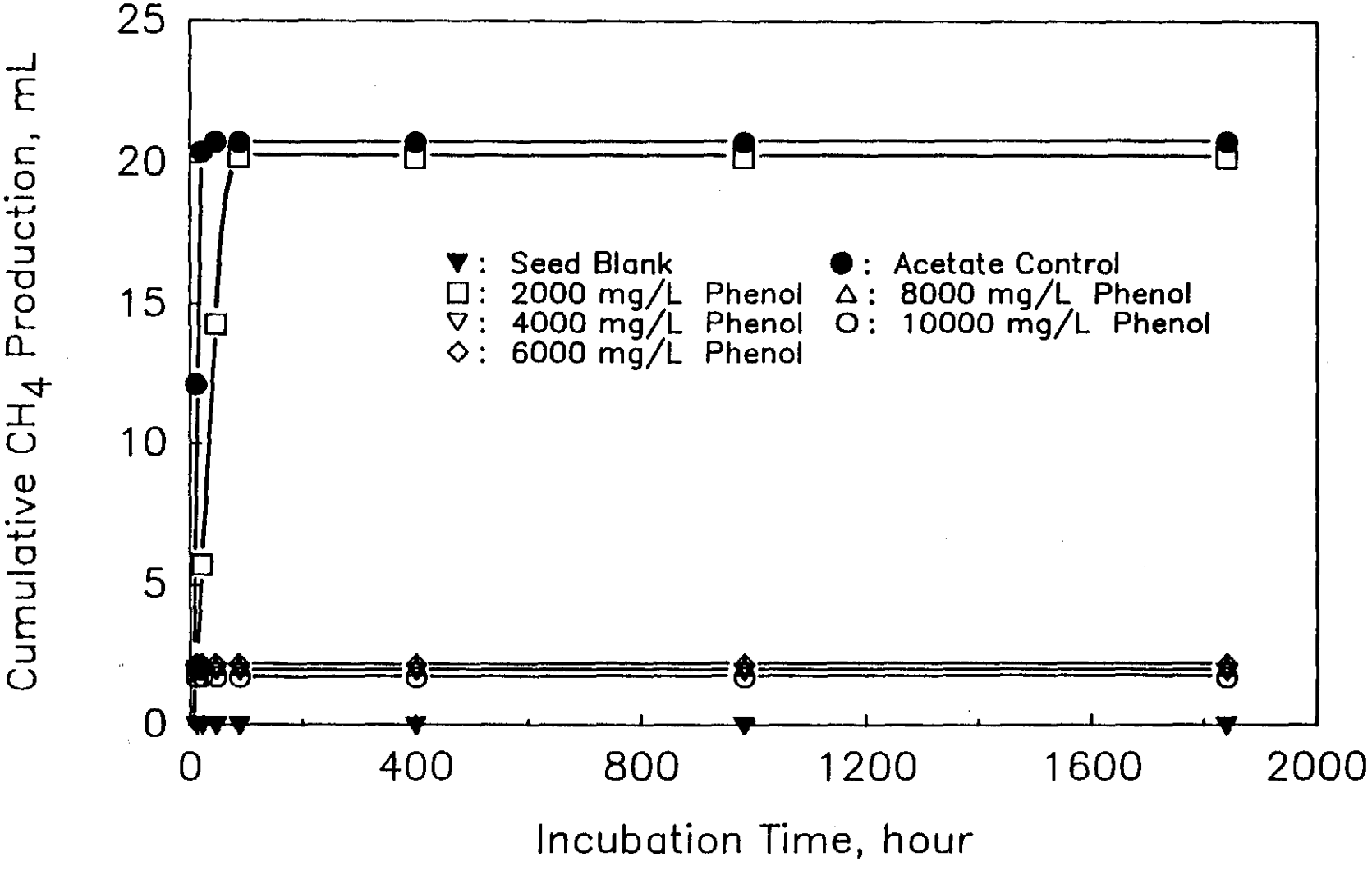

Figure 20. Methane Production from Added Acetate in the Presence of Phenol: Higher Concentrations

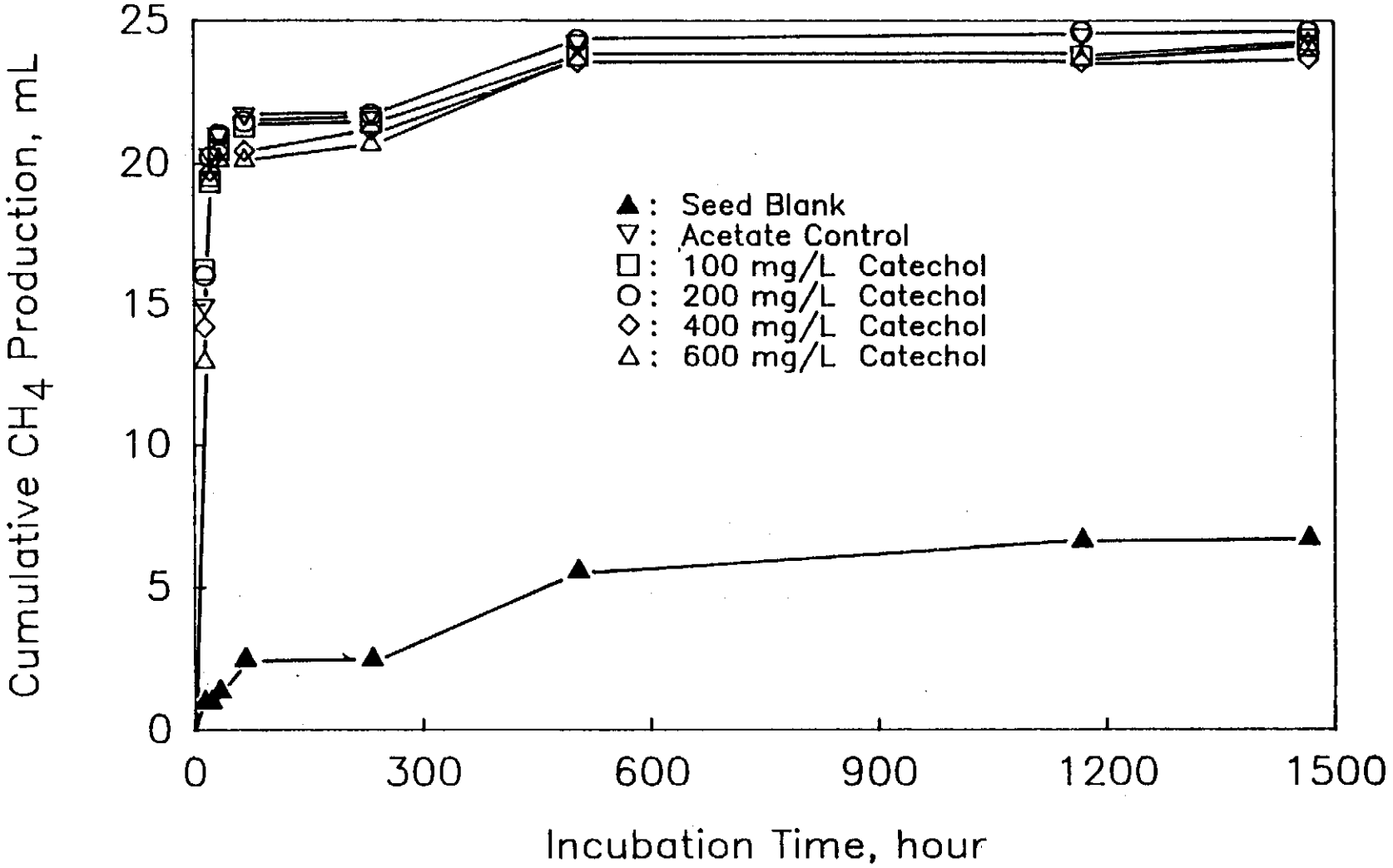

Figure 21. Methane Production from Added Acetate in the Presence of Catechol: Low Concentrations

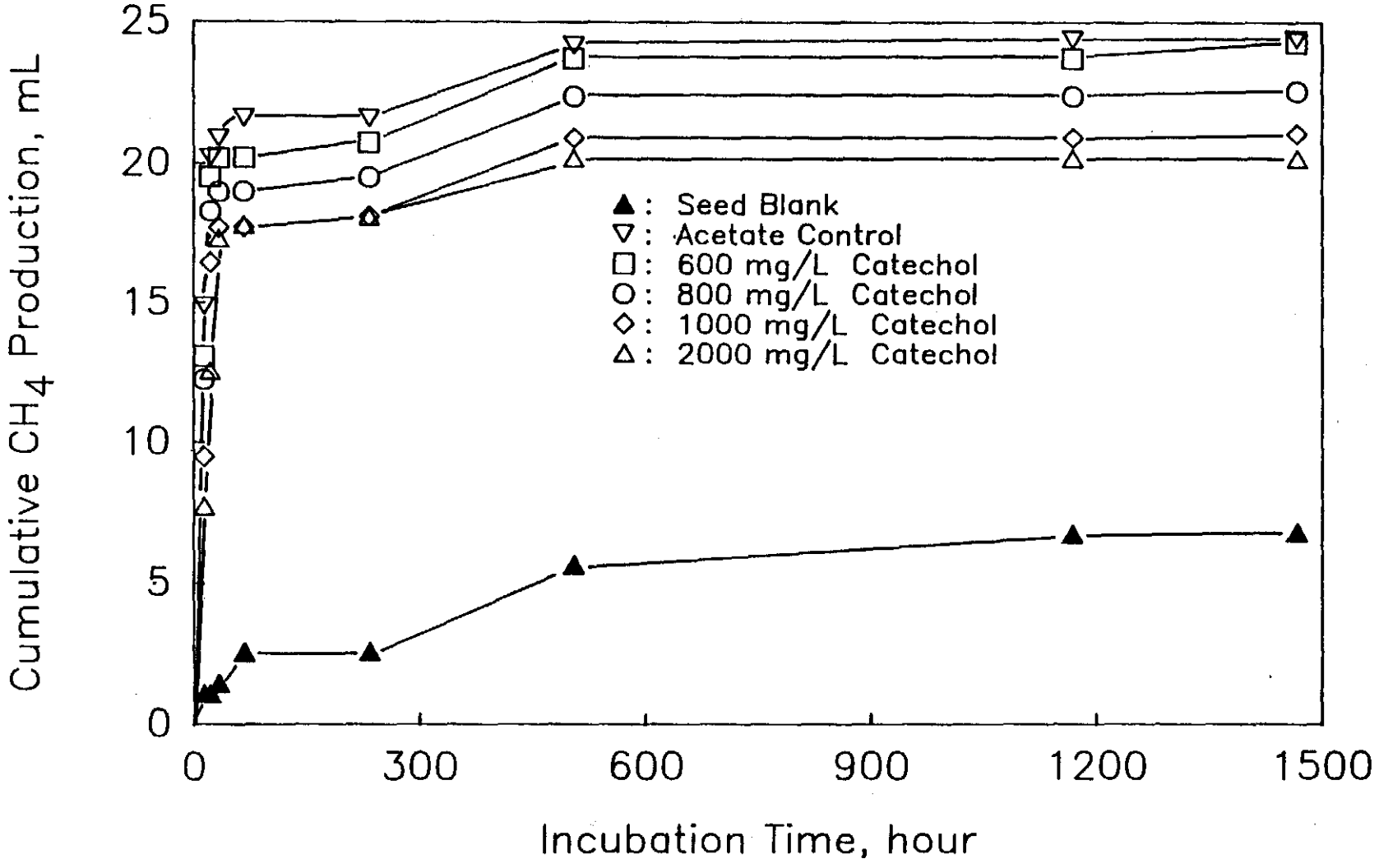

Figure 22. Methane Production from Added Acetate in the Presence of Catechol: Intermediate Concentrations

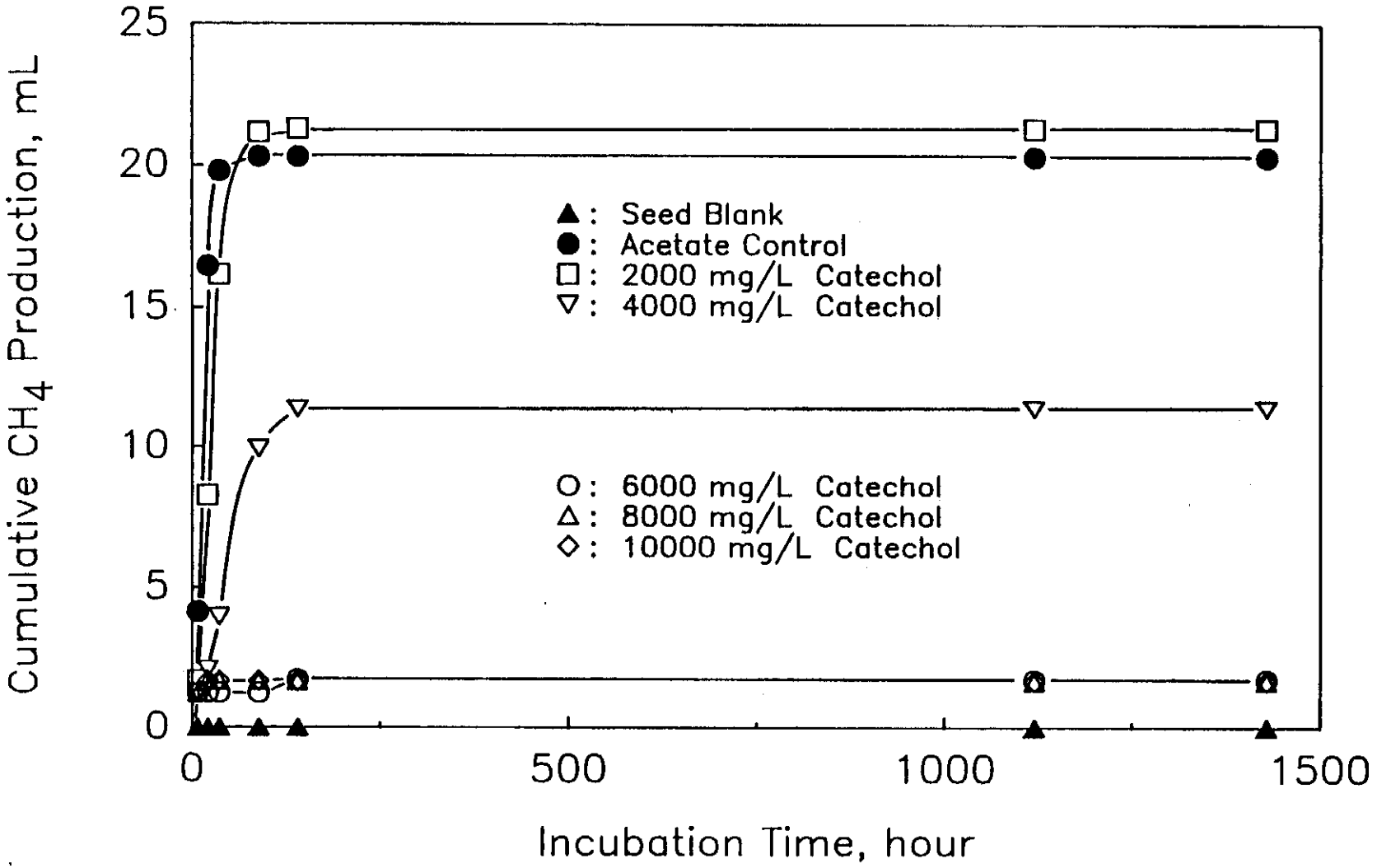

Figure 23. Methane Production from Added Acetate in the Presence of Catechol: High Concentrations

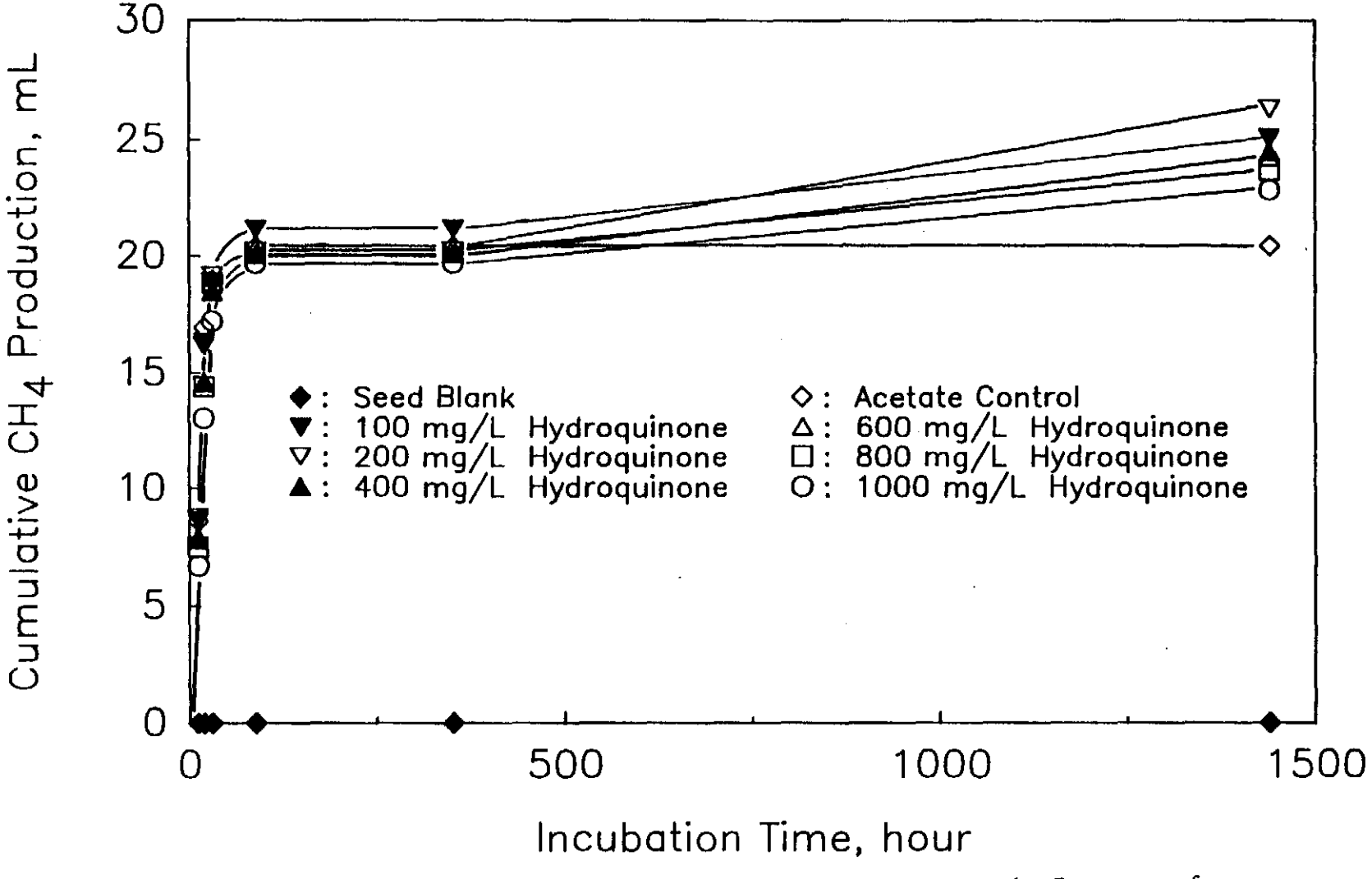

Figure 24. Methane Production from Added Acetate in the Presence of Hydroquinone: Lower Concentrations

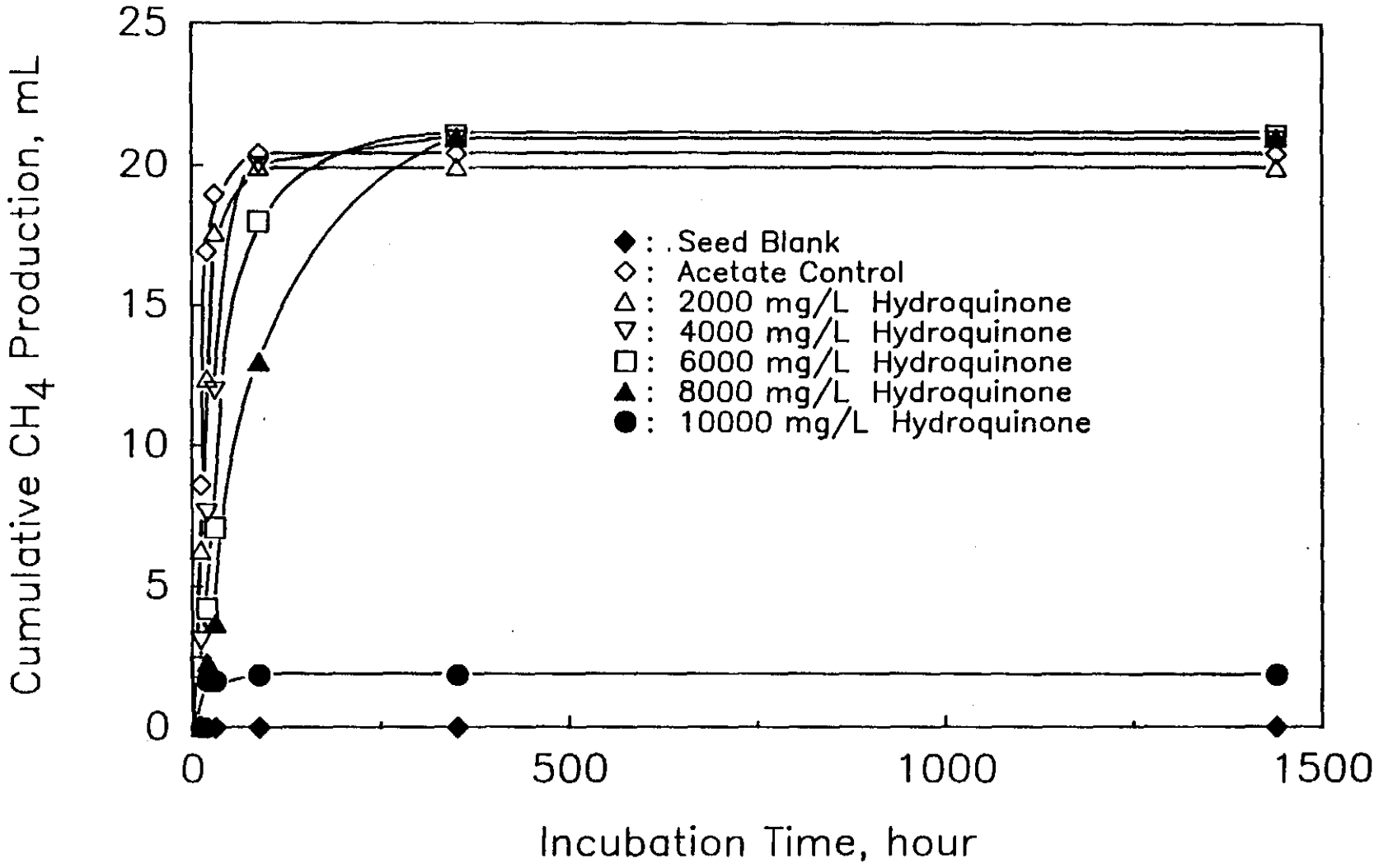

Figure 25. Methane Production from Added Acetate in the Presence of Hydroquinone: Higher Concentrations

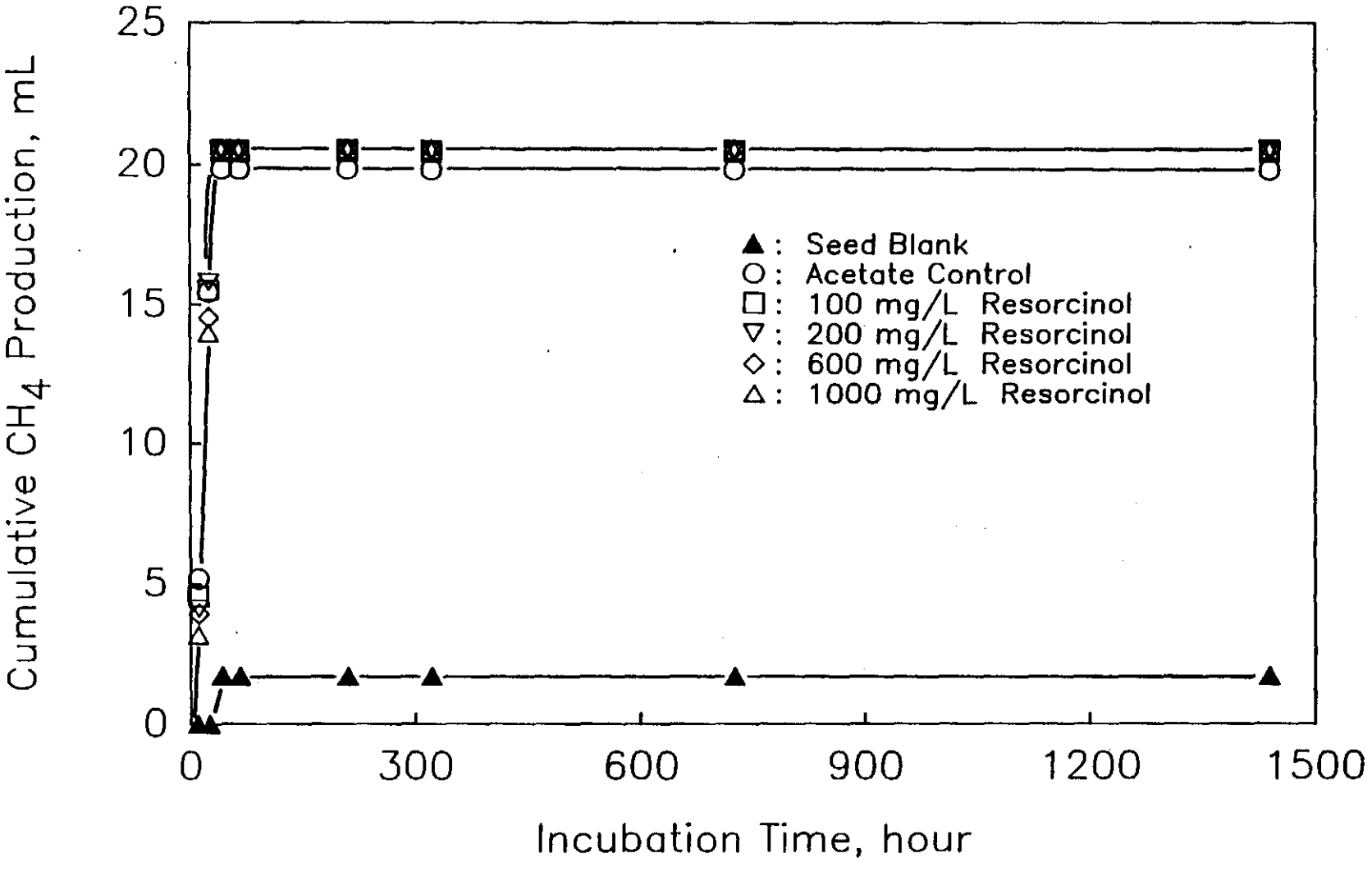

Figure 26. Methane Production from Added Acetate in the Presence of Resorcinol: Low Concentrations

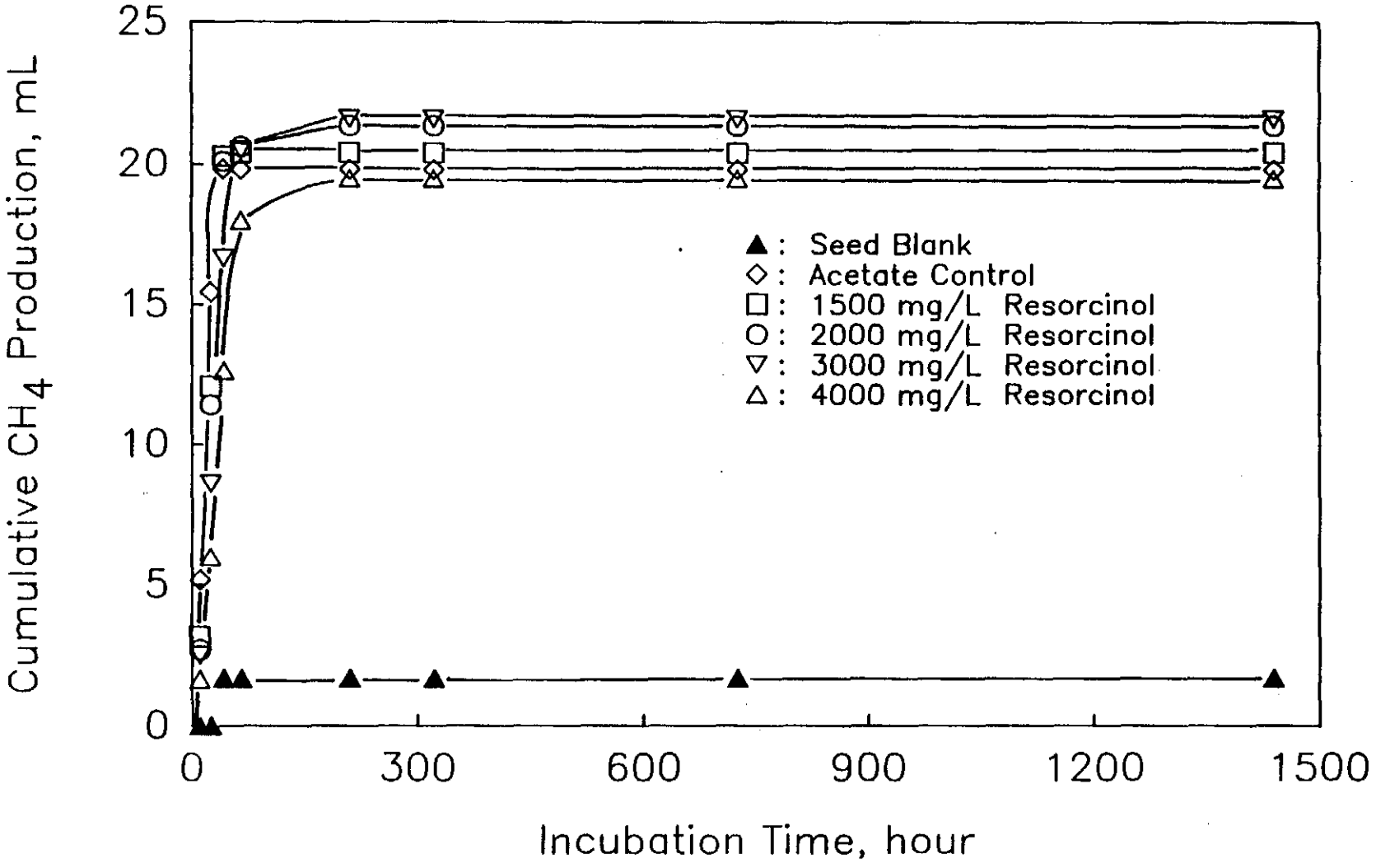

Figure 27. Methane Production from Added Acetate in the Presence of Resorcinol: Intermediate Concentrations

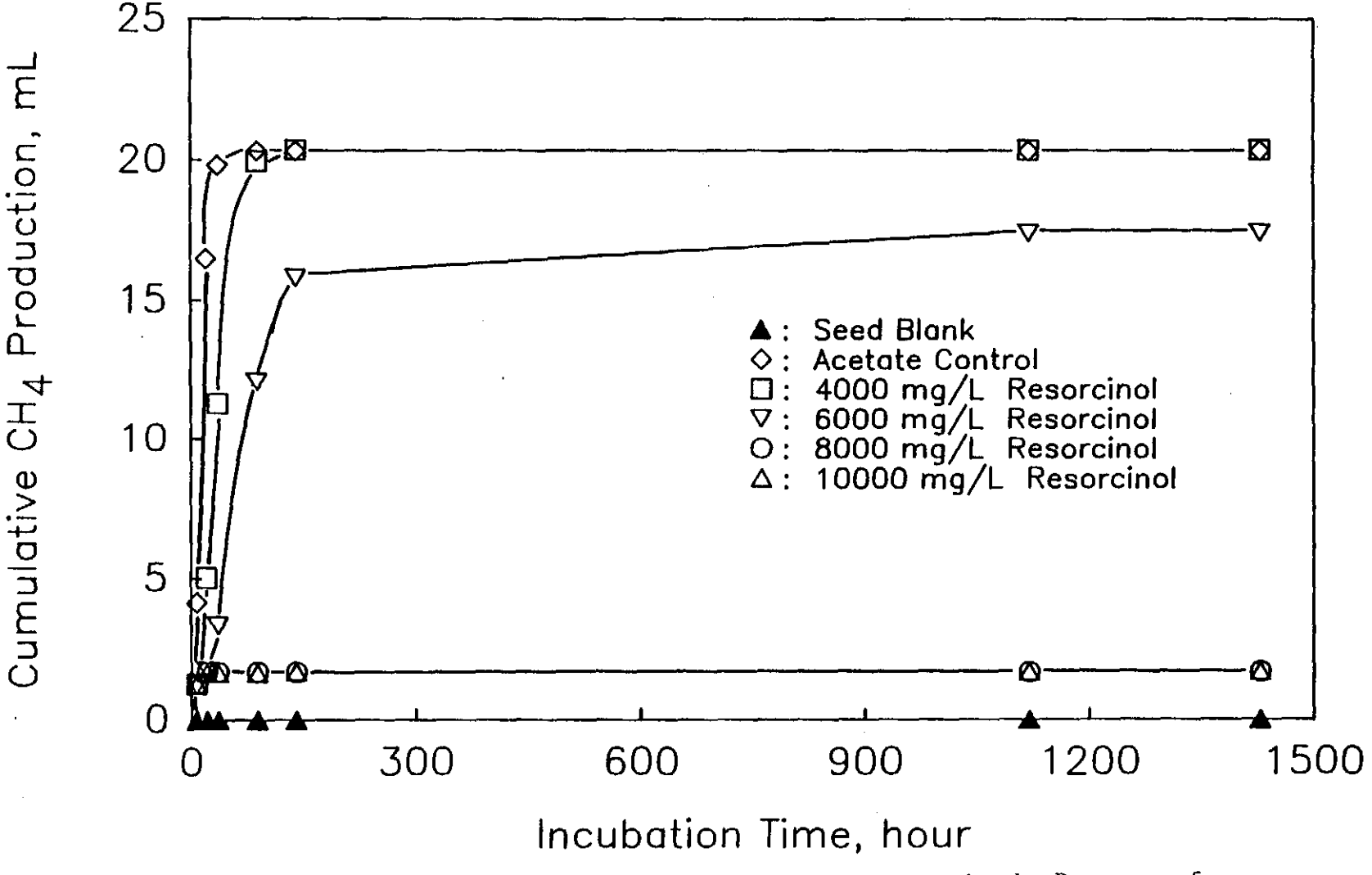

Figure 28. Methane Production from Added Acetate in the Presence of Resorcinol: High Concentrations

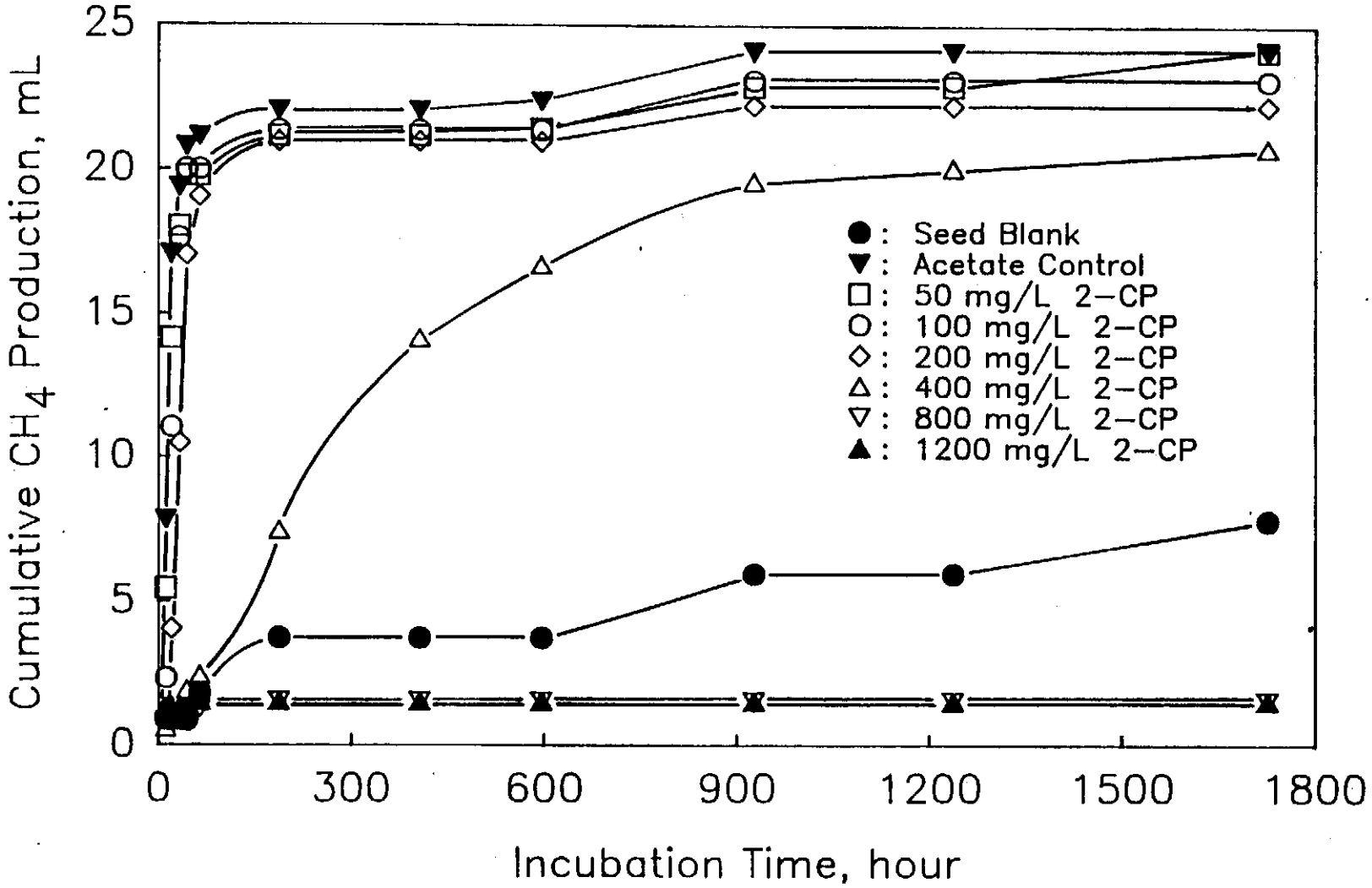

Figure 29. Methane Production from Added Acetate in the Presence of 2-CP

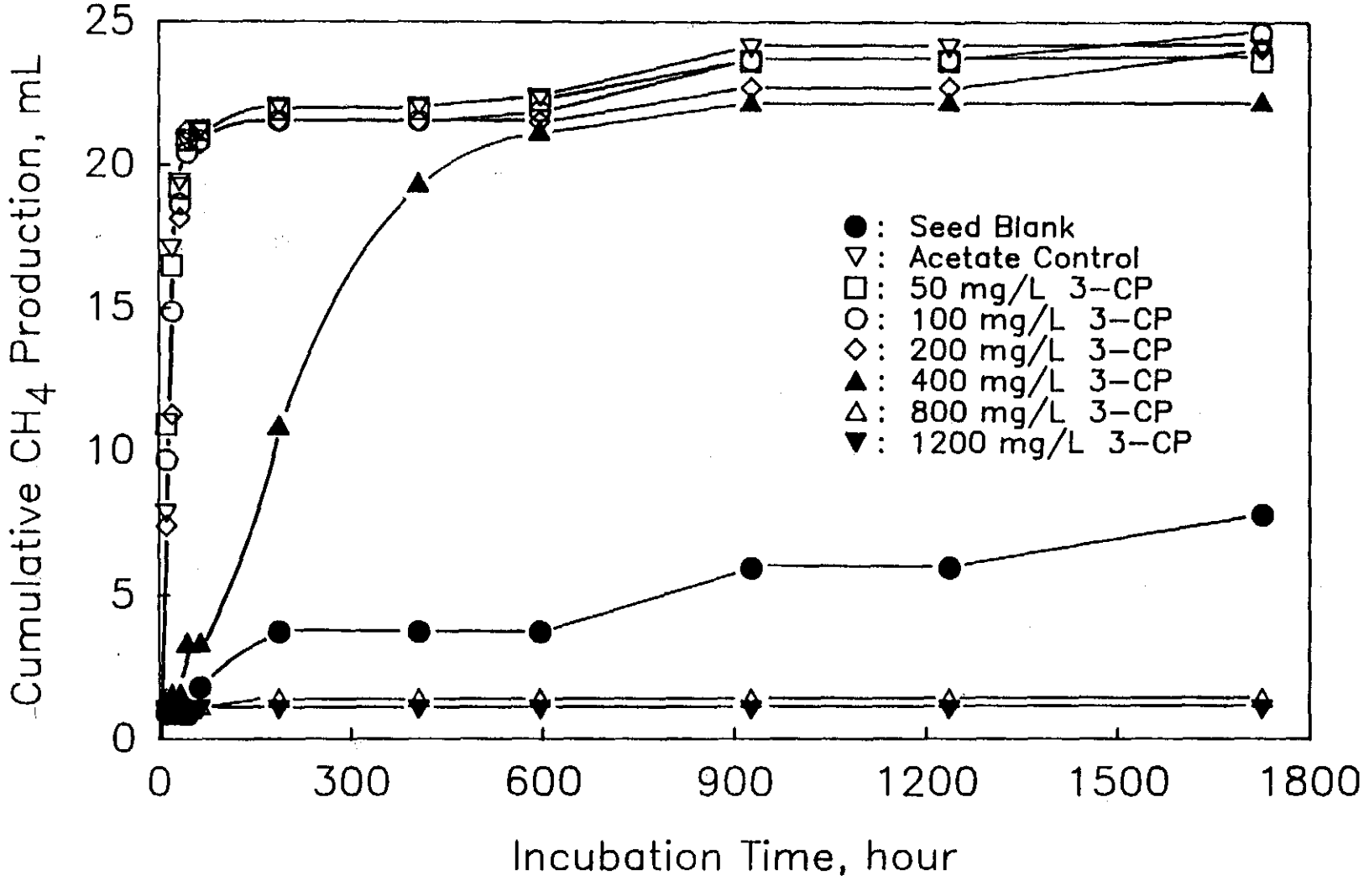

Figure 30. Methane Production from Added Acetate in the Presence of 3-CP

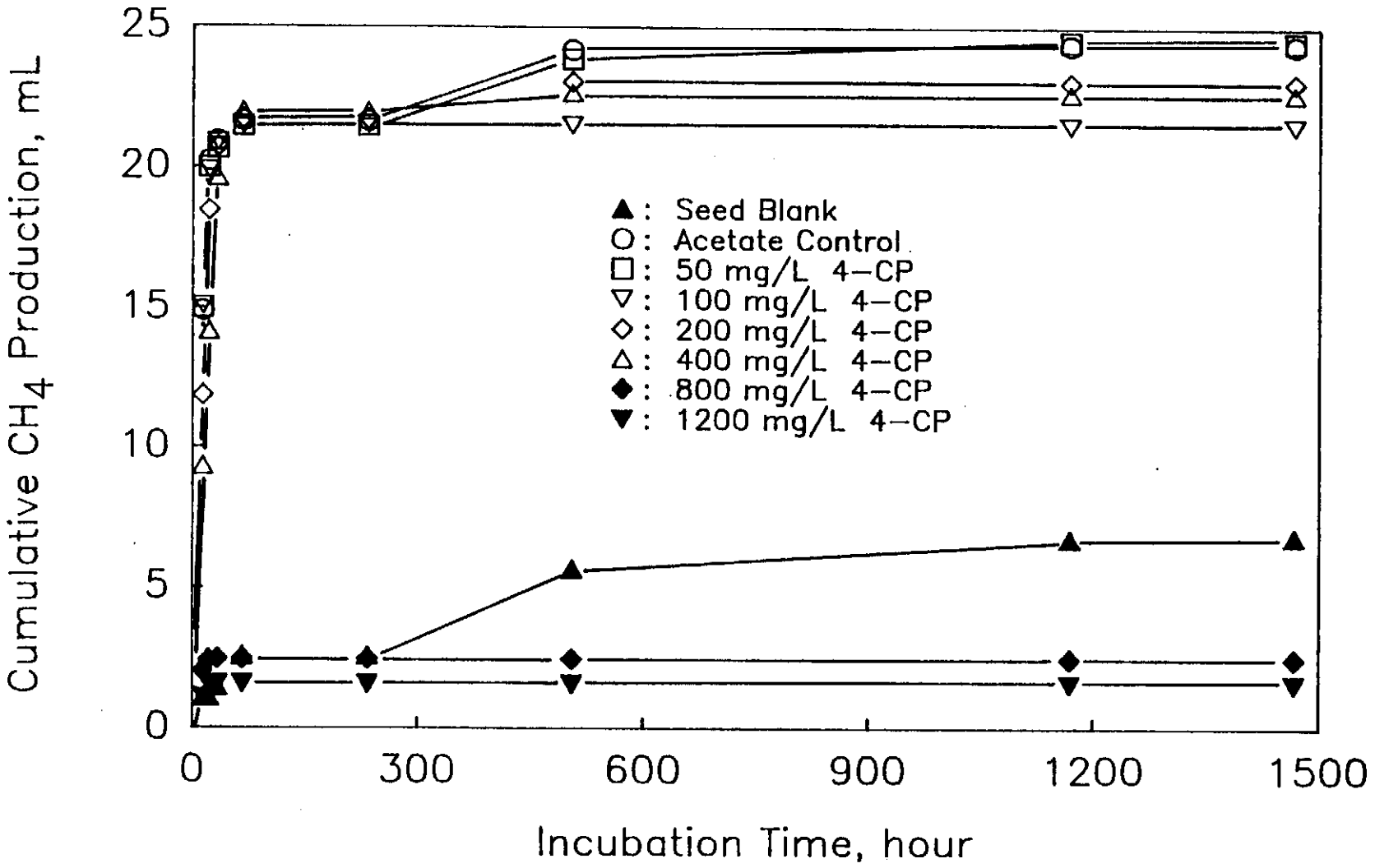

Figure 31. Methane Production from Added Acetate in the Presence of 4-CP

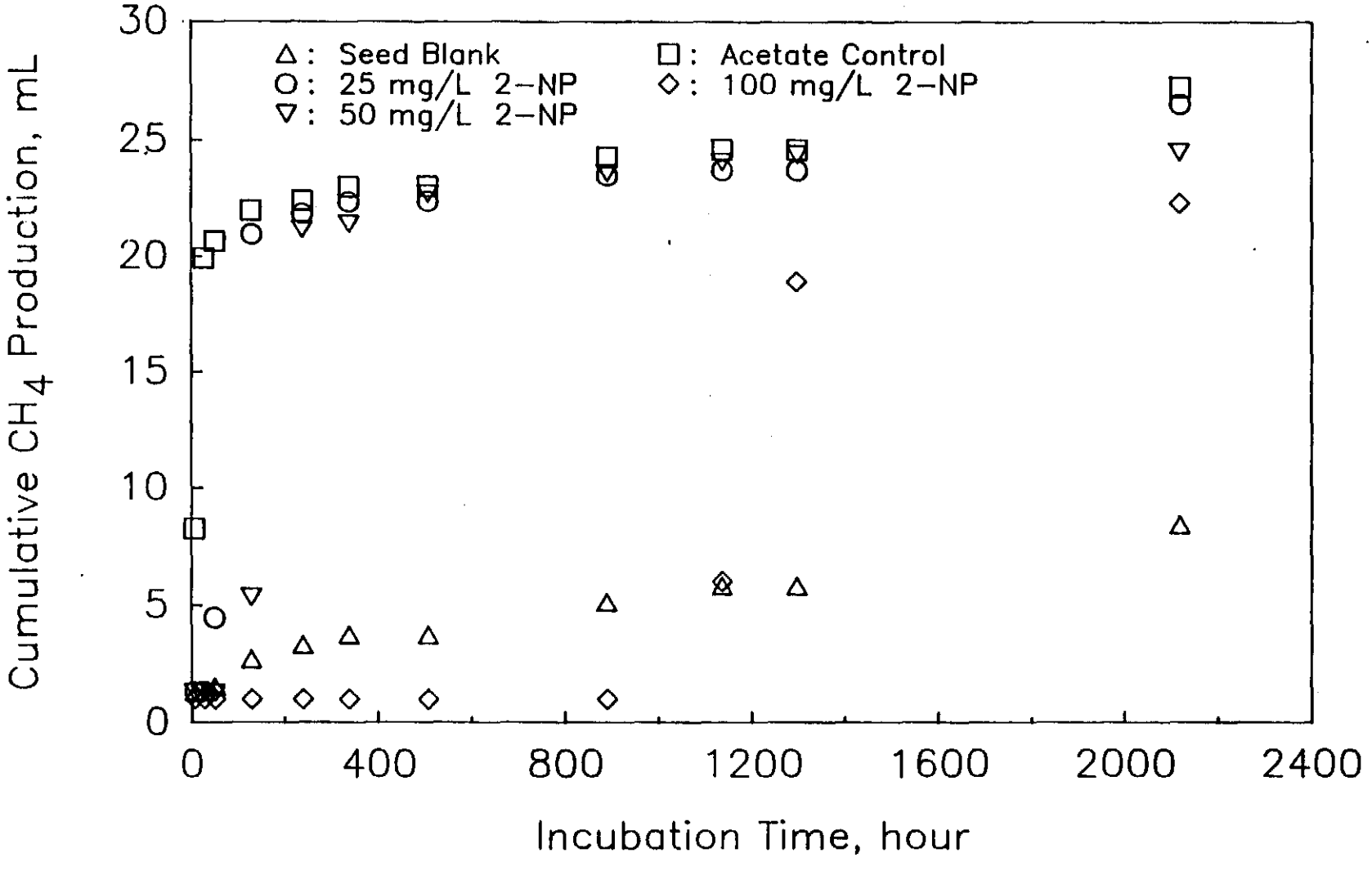

Figure 32. Methane Production from Added Acetate in the Presence of 2-NP: Lower Concentrations

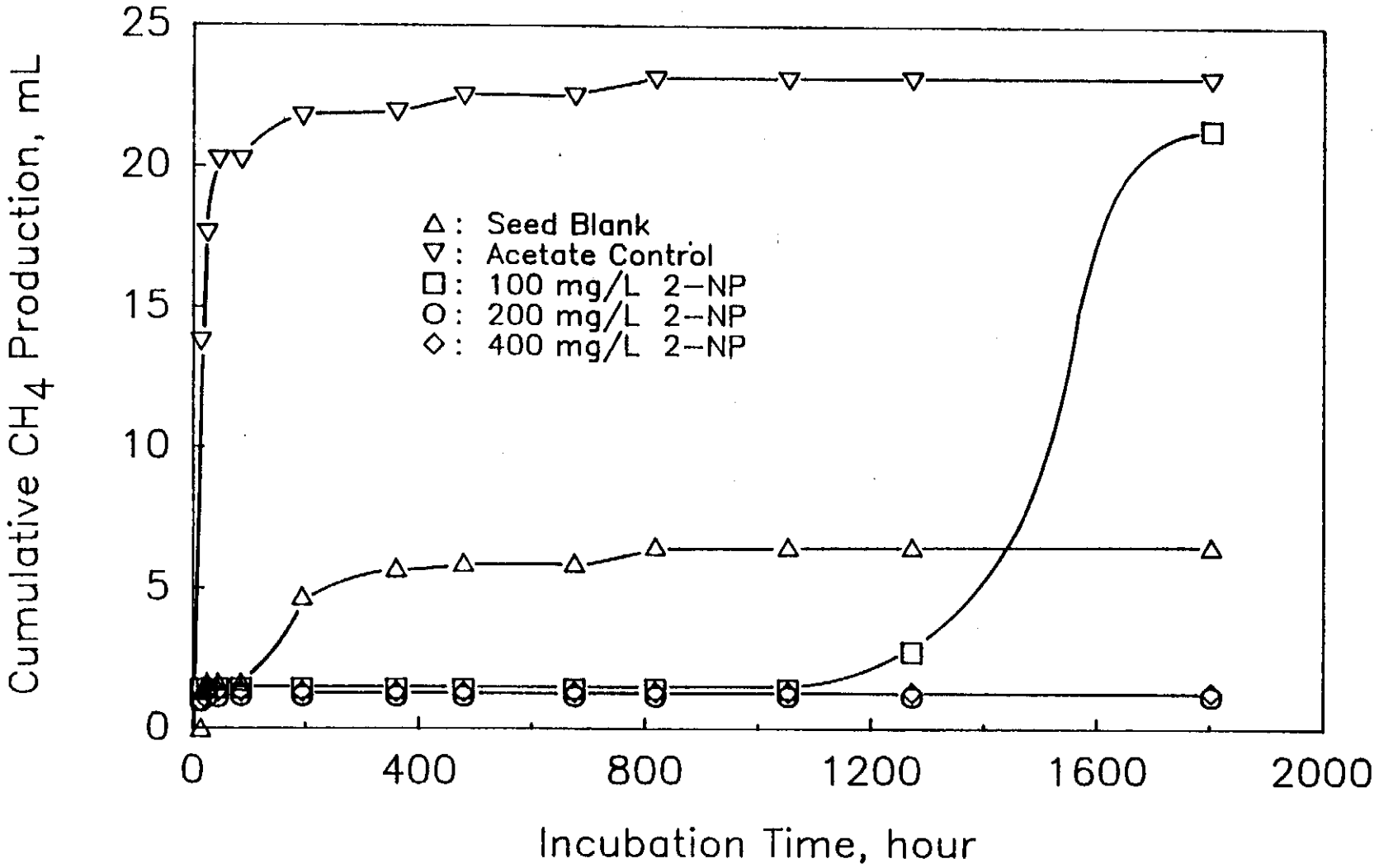

Figure 33. Methane Production from Added Acetate in the Presence of 2-NP: Higher Concentrations

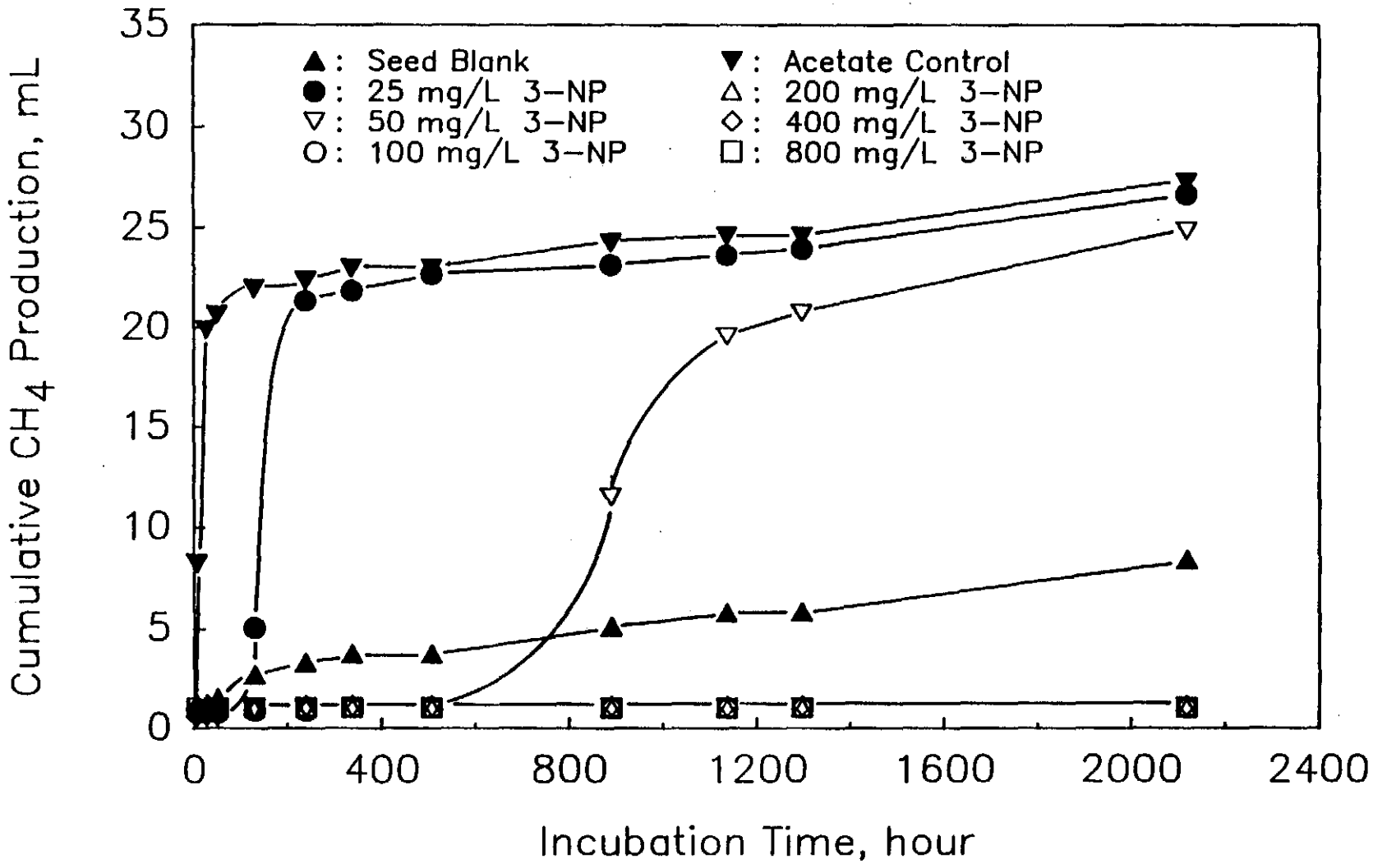

Figure 34. Methane Production from Added Acetate in the Presence of 3-NP

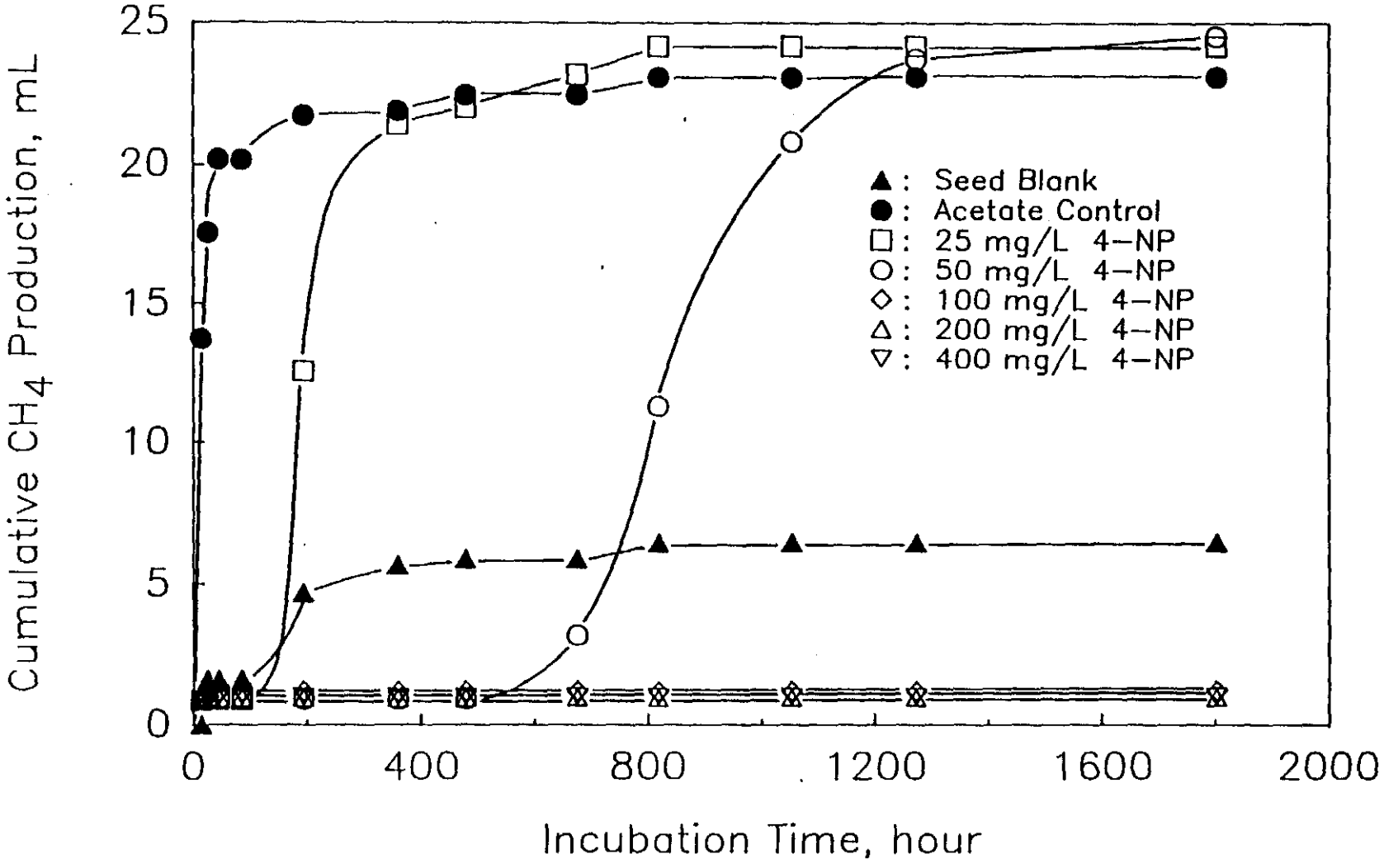

Figure 35. Methane Production from Added Acetate in the Presence of 4-NP

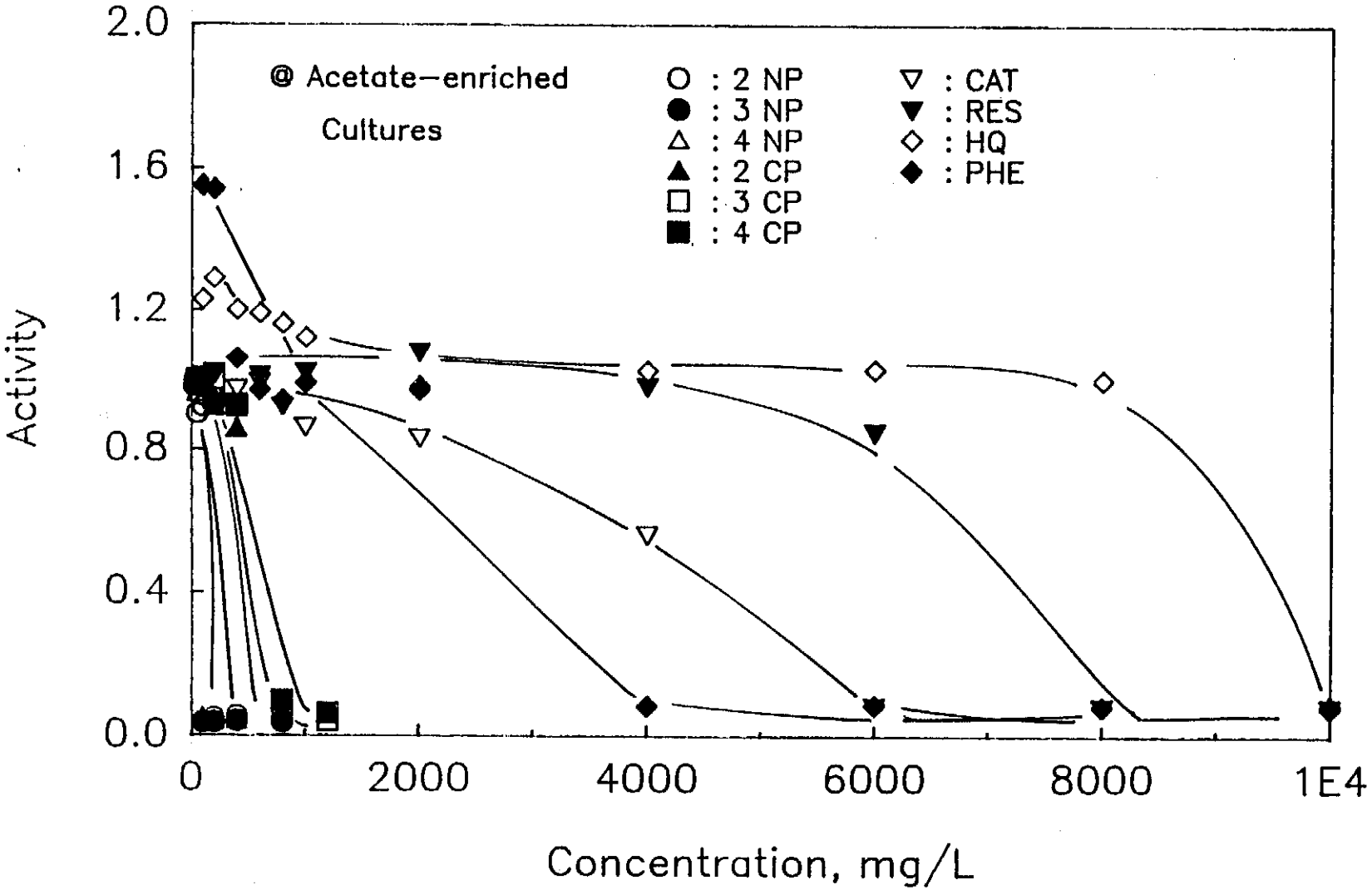

Figure 36. Effect of Phenolic Concentration on Methanogenic Activity in the Acetate-enriched Culture

Table 9 Toxicity Levels of Phenols for Acetate-Utilizing Methanogens

Compound	Concentration corresponding to 50% activity reduction, mg/ℓ	Concentration corresponding to no activities, mg/ℓ
Phenol	2500	4000
Catechol	4000	6000
Hydroquinone	9300	10000
Resorcinol	7000	8000
2-CP	600	800
3-CP	650	800
4-CP	650	800
2-NP	100	200
3-NP	140	100
4-NP	140	100

200 mg/L after two months incubation. All other phenolic compounds were not significantly degraded in the acetate-enriched culture even at very low concentrations (Tables 10-13).

Inhibition of Phenol Degradation

In order to asses the inhibitory nature of the substituted phenols toward the aromatic ring degrading bacteria, batch toxicity assay was conducted with phenol as the substrate. The patterns of phenol degradation and concomitant methane production in the presence of a substituted phenol are shown in Figures 37-55. The total methane data from Figures 37-46 are expressed as methanogenic activity as plotted in Figure 56. The effects of substitution groups on phenol degradation are similar to those found with the acetate-enriched culture. The nitro group was the most inhibitory while the hydroxyl group was the least toxic. Both the methane data (Figure 56 and Table 14) and the phenol data (Figures 47-55) indicated that the observed toxicity levels were 50, 100-200, and 400-1000 mg/L for nitro, chloro, and hydroxyphenols, respectively. These levels are lower than those observed with the acetate-enriched culture. In addition, no significant acid accumulation was observed when phenol degradation was inhibited. Thus, the phenol degraders were more susceptible to inhibition by the substituted phenols than the acetate-utilizing methanogens. Similar findings were reported earlier with some alkyl phenols (Wang, et al., 1988).

Except catechol, the effects on phenol degradation did not differ significantly among the isomers. In contrast to findings with the acetate-enriched culture, in which catechol was the most inhibitory among the hydroxyphenols, catechol was far less inhibitory than the other two isomers. Since the phenol-enriched culture had been maintained on a feed solution containing catechol for more than two years, the observed lower toxicity

Table 10 Biodegradation Potential of Phenol in the Acetate-enriched Culture

Compound	Spiked concentration, mg/ℓ nominal	Spiked concentration, mg/ℓ measured	Final concentration, mg/ℓ
Phenol	100	103	—
	200	185	50
	400	360	300
	600	520	440
	800	730	690
	1,000	830	860
	2,000	1815	1930
	4,000	3330	3500
	6,000	4930	5370
	8,000	6740	6940
	10,000	8330	8400

*incubated for 1840 hr

Table 11 Biodegradation Potential of Hydroxyphenols in the Acetate-enriched Culture

Compound	Spiked concentration, mg/ℓ Nominal	measured	Final Concentration, mg/ℓ	Incubation period, hour
Catchol	100	98	100	1440
	200	213	208	1440
	400	478	430	1440
	600	663	601	1440
	800	919	872	1440
	1,000	1114	1052	1440
	2,000	1980	2090	1440
	4,000	4078	4065	1440
	6,000	6078	6160	1429
	8,000	8048	7880	1429
	10,000	10310	9970	1429
Hydroquinone	100	—	24	
	200	—	110	
	400	—	275	
	600	—	565	1440
	800	—	820	
	1,000	—	1055	
	2,000	2040	2180	
	4,000	3970	4405	
	6,000	6200	6915	
	8,000	7625	8660	
	10,000	10600	10925	
Resorcinol	100	80	73	1440
	200	153	153	1440
	600	420	475	1440
	1,000	930	770	1440
	1,500	1435	1390	1440
	2,000	1790	1975	1440
	3,000	2810	2805	1440
	4,000	3675	3763	1440
	6,000	5620	5788	1429
	8,000	7740	7838	1429
	10,000	9510	9650	1429

Table 12 Biodegradation Potential of Chlorophenols in the Acetate-enriched Culture

Compound	Spiked concentration, mg/ℓ nominal	Spiked concentration, mg/ℓ measured	Final concentration, mg/ℓ	Incubation period, hour
2-CP	50	55	58	
	100	107	118	
	200	216	235	1727
	400	465	455	
	800	770	798	
	1200	944	1108	
3-CP	50	40	46	
	100	69	66	
	200	135	126	1727
	400	268	250	
	800	560	500	
	1200	833	794	
4-CP	50	—	49	
	100	—	86	
	200	—	169	1468
	400	271	307	
	800	608	608	
	1200	999	960	

Table 13 Biodegradation Potential of Nitrophenols in the Acetate-enriched Culture

Compound	Spiked concentration, mg/ℓ nominal	Spiked concentration, mg/ℓ measured	Final concentration mg/ℓ	Incubation period, hour
2-NP	25	—	2	2119
	50	—	15	2119
	100	69	0	1803
	200	140	35	1803
	400	250	95	1803
3-NP	25	—	23	
	50	—	59	
	100	—	130	2119
	200	186	181	
	400	375	434	
	800	730	820	
4-NP	25	17	0.5	
	50	48	1.5	1803
	100	119	23	
	200	402	288	
	400	535	394	

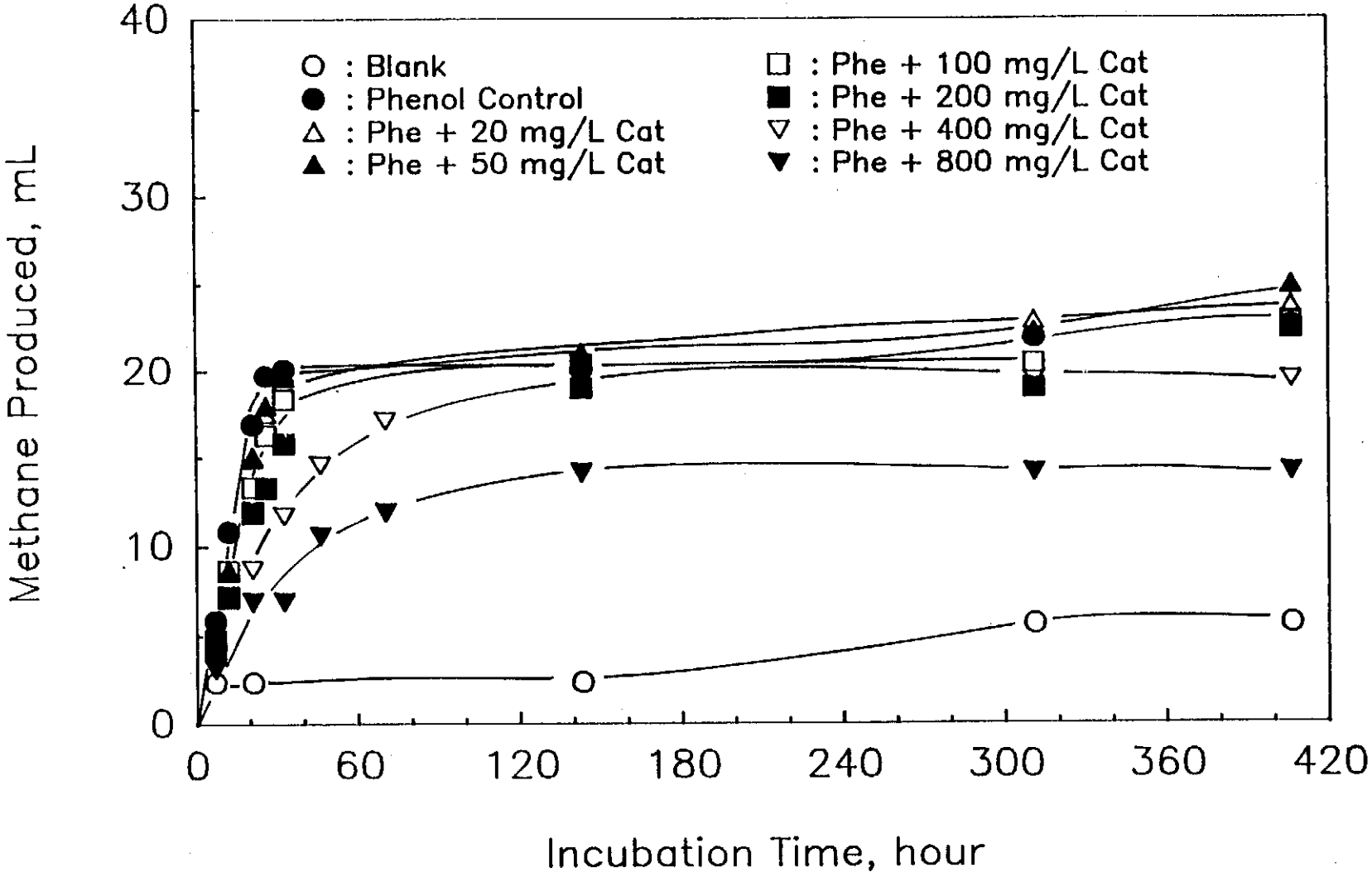

Figure 37. Methane Production from Phenol-supplemented Culture in the Presence of Catechol: Early Stages

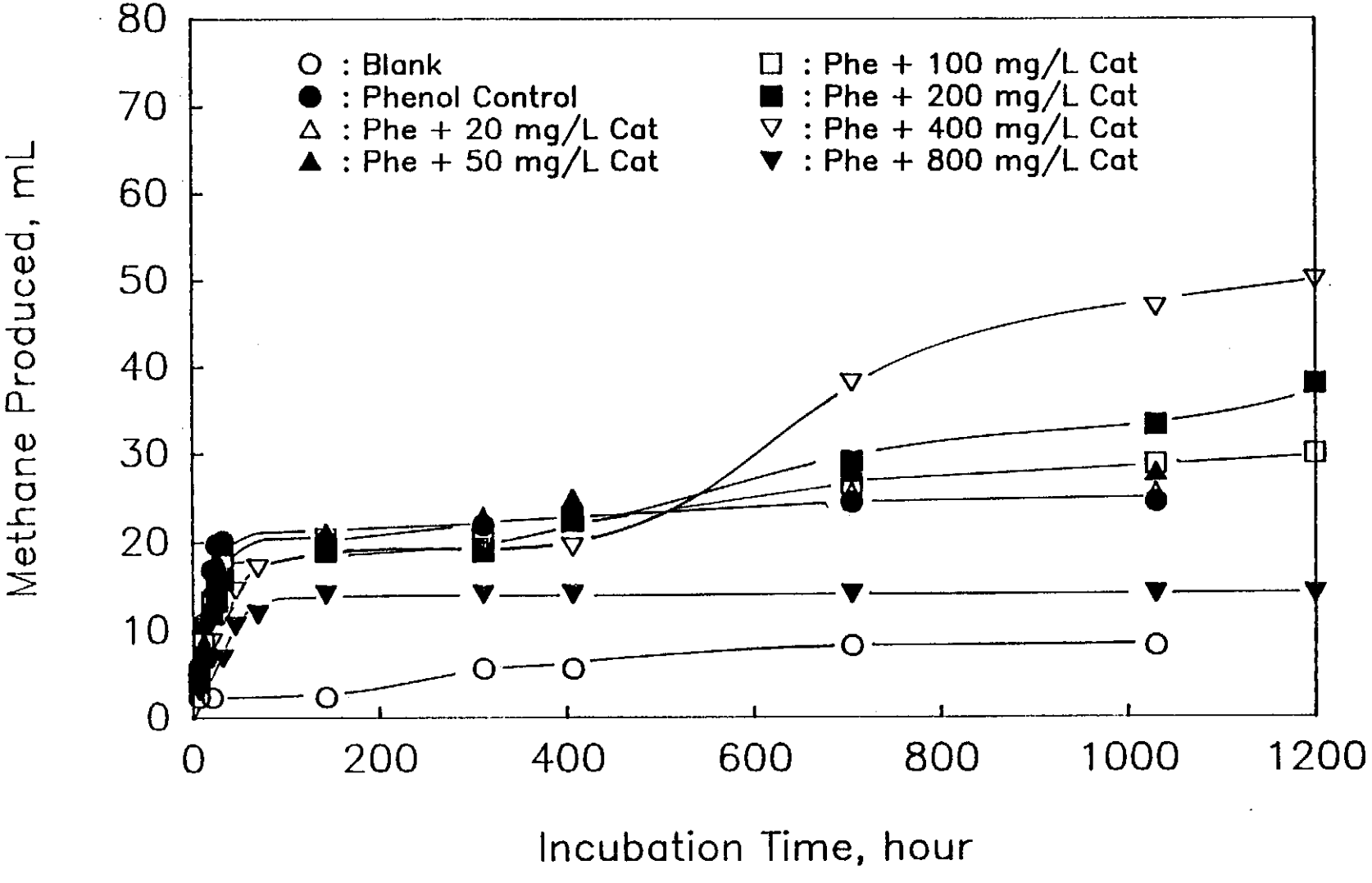

Figure 38. Methane Production from Phenol-supplemented Culture in the Presence of Catechol: Entire Incubation

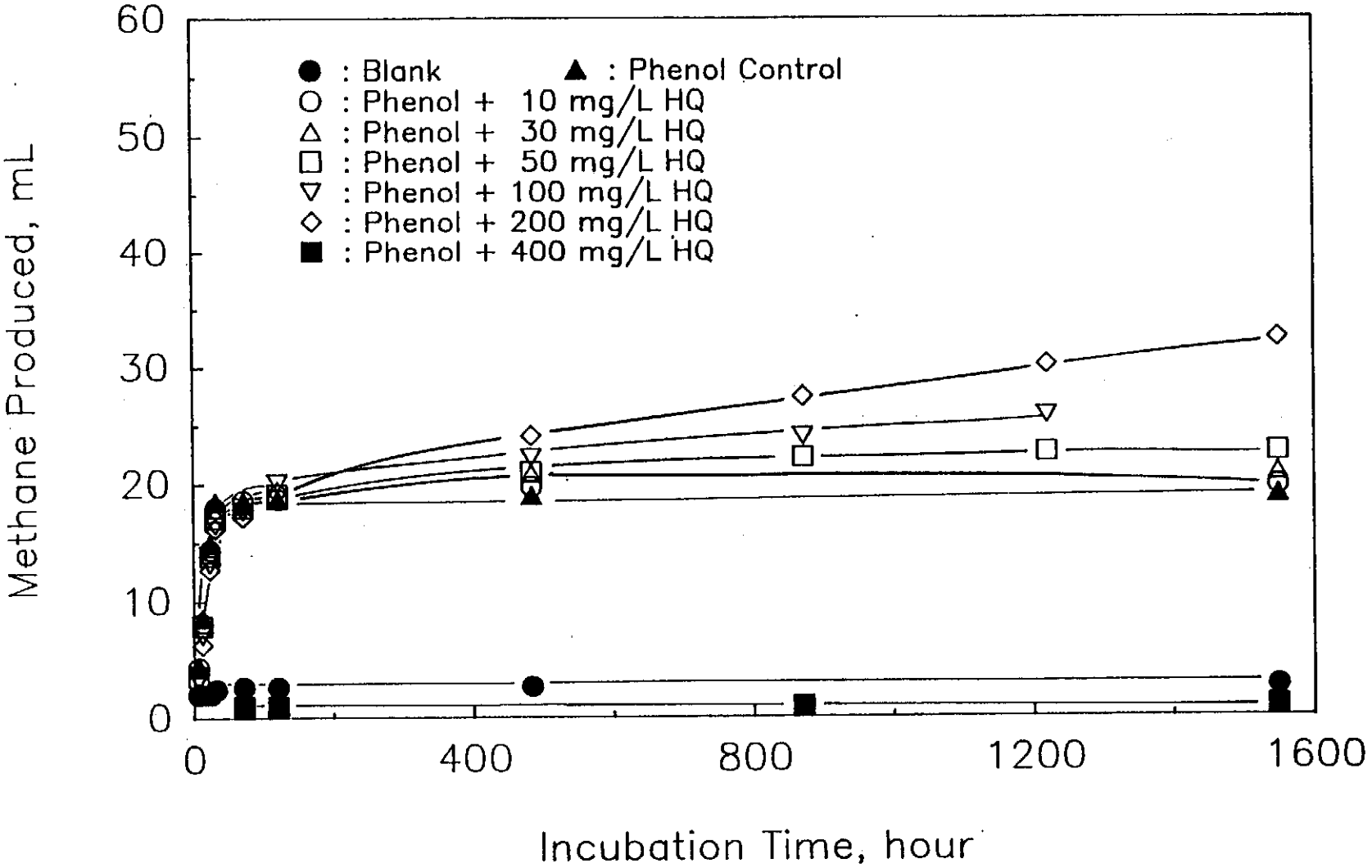

Figure 39. Methane Production from Phenol-supplemented Culture in the Presence of Hydroquinone

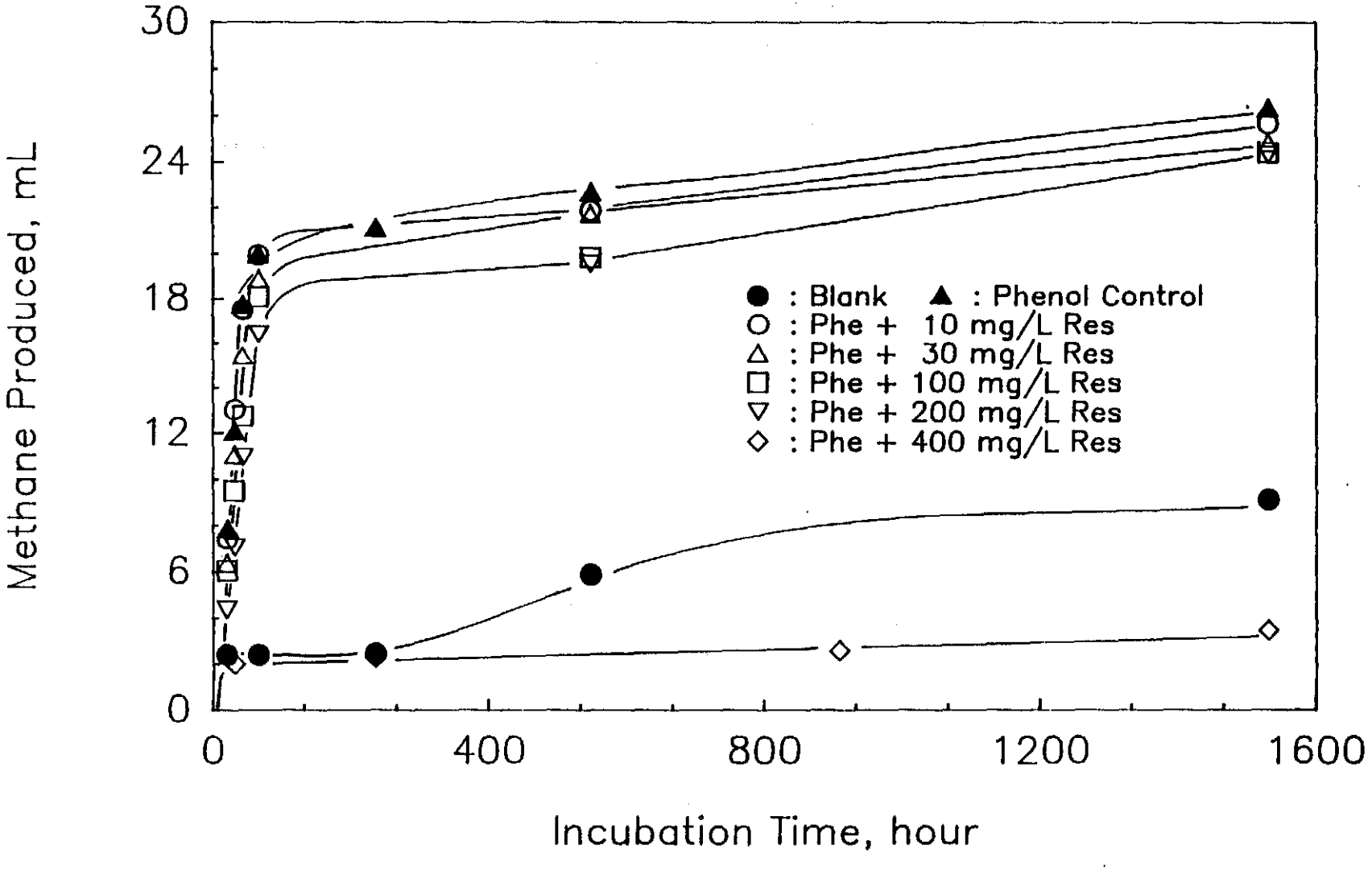

Figure 40. Methane Production from Phenol-supplemented Culture in the Presence of Resorcinol

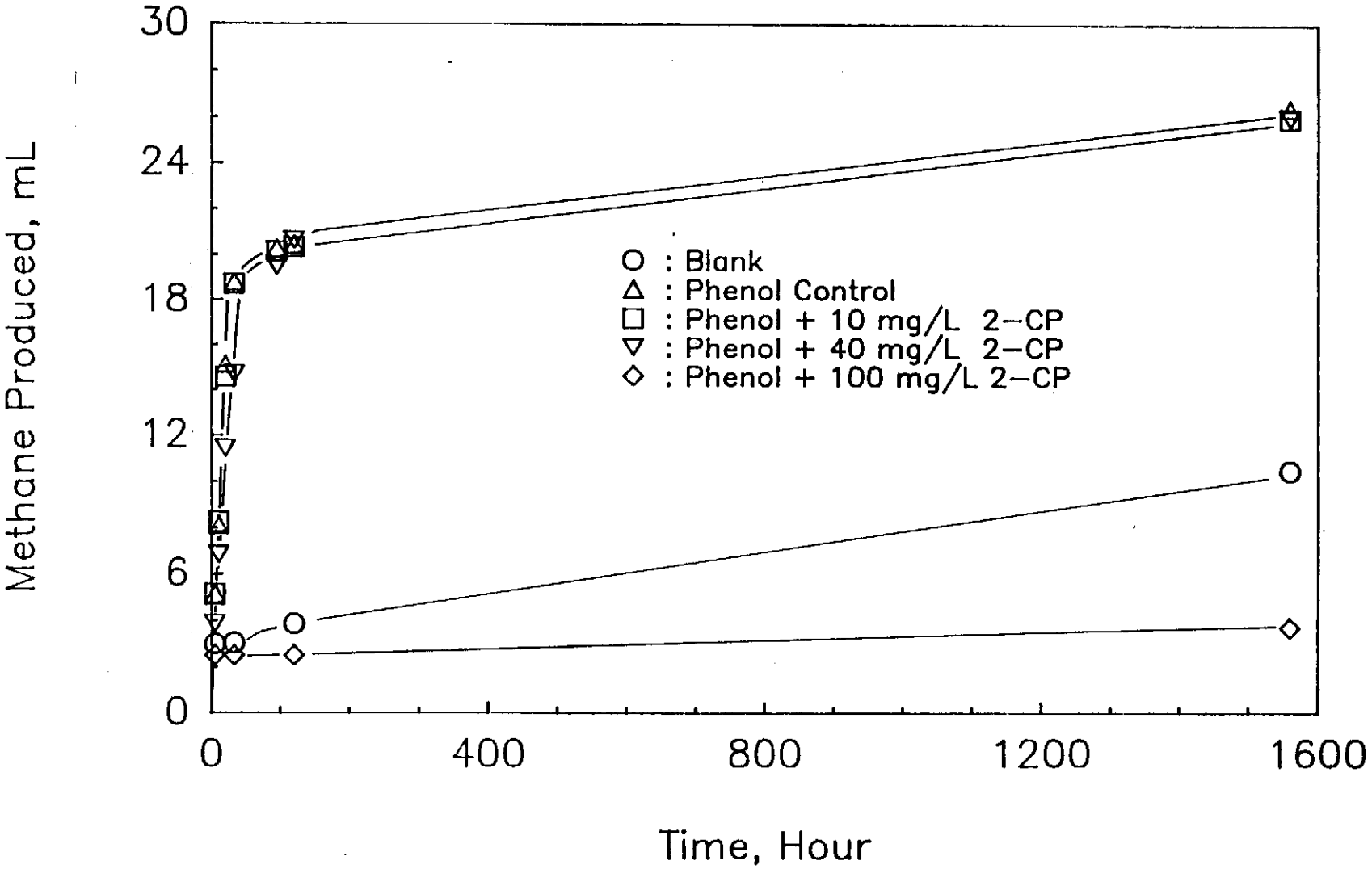

Figure 41. Methane Production from Phenol-supplemented Culture in the Presence of 2-CP

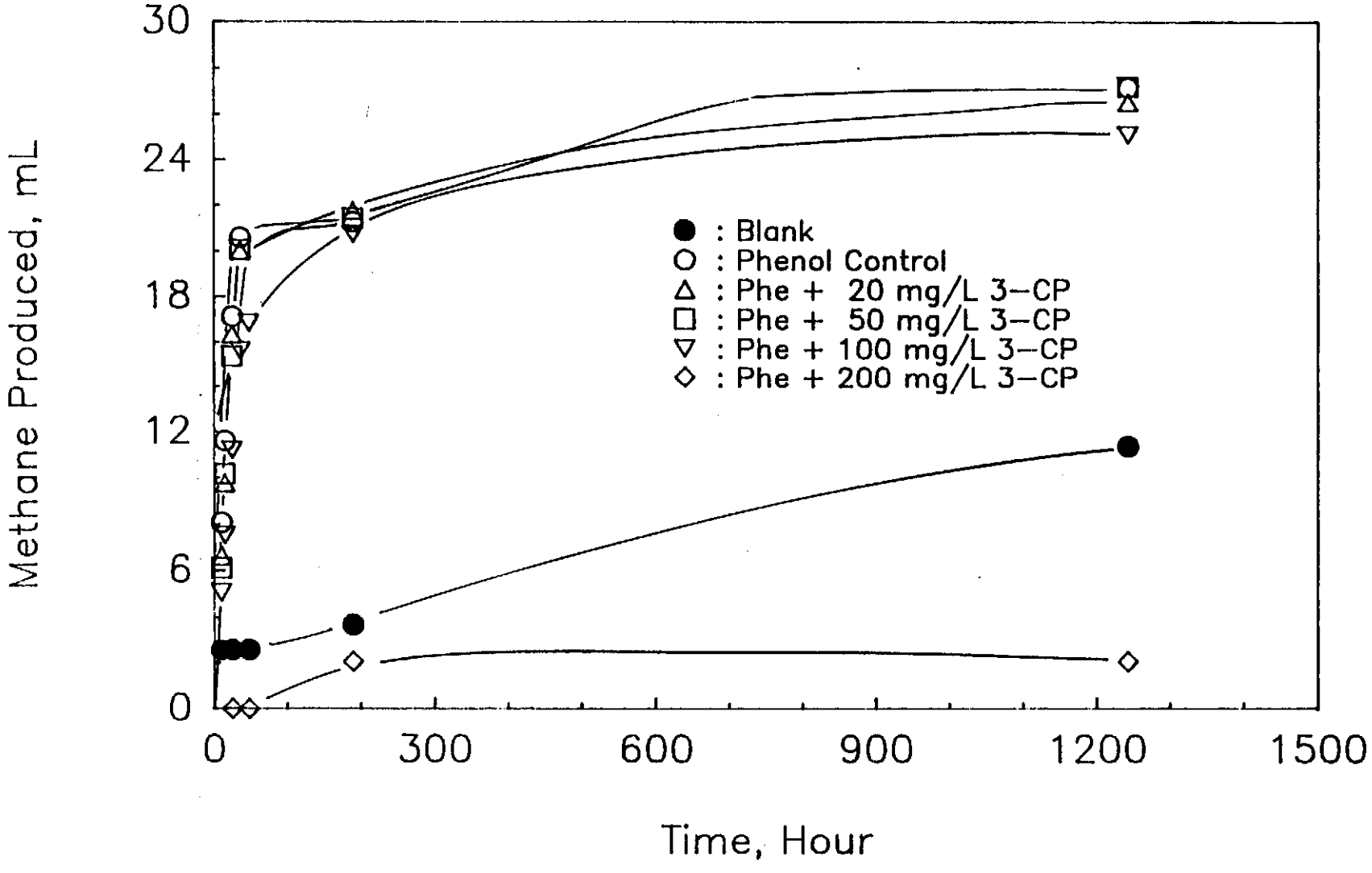

Figure 42. Methane Production from Phenol-supplemented Culture in the Presence of 3-CP

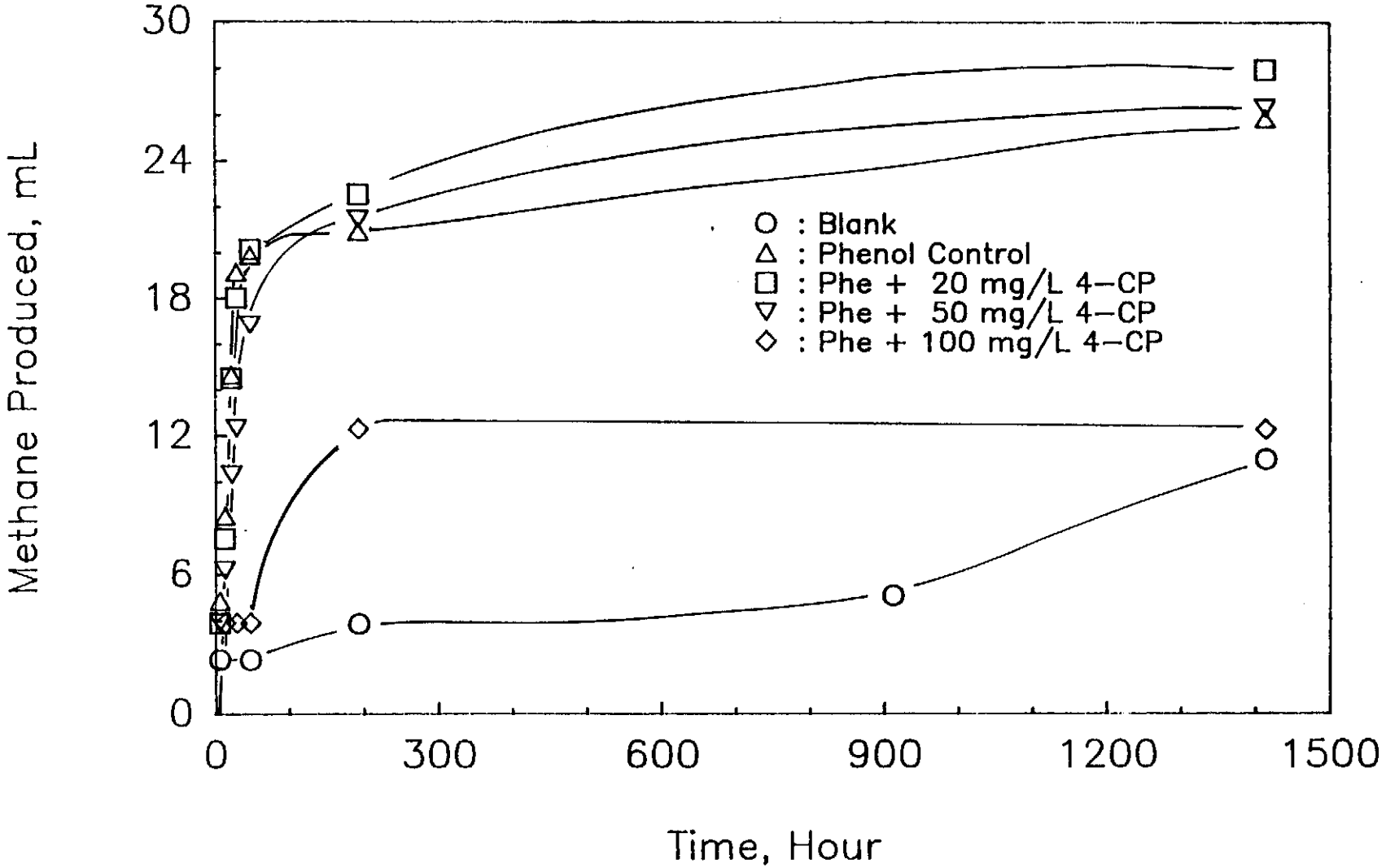

Figure 43. Methane Production from Phenol-supplemented Culture in the Presence of 4-CP

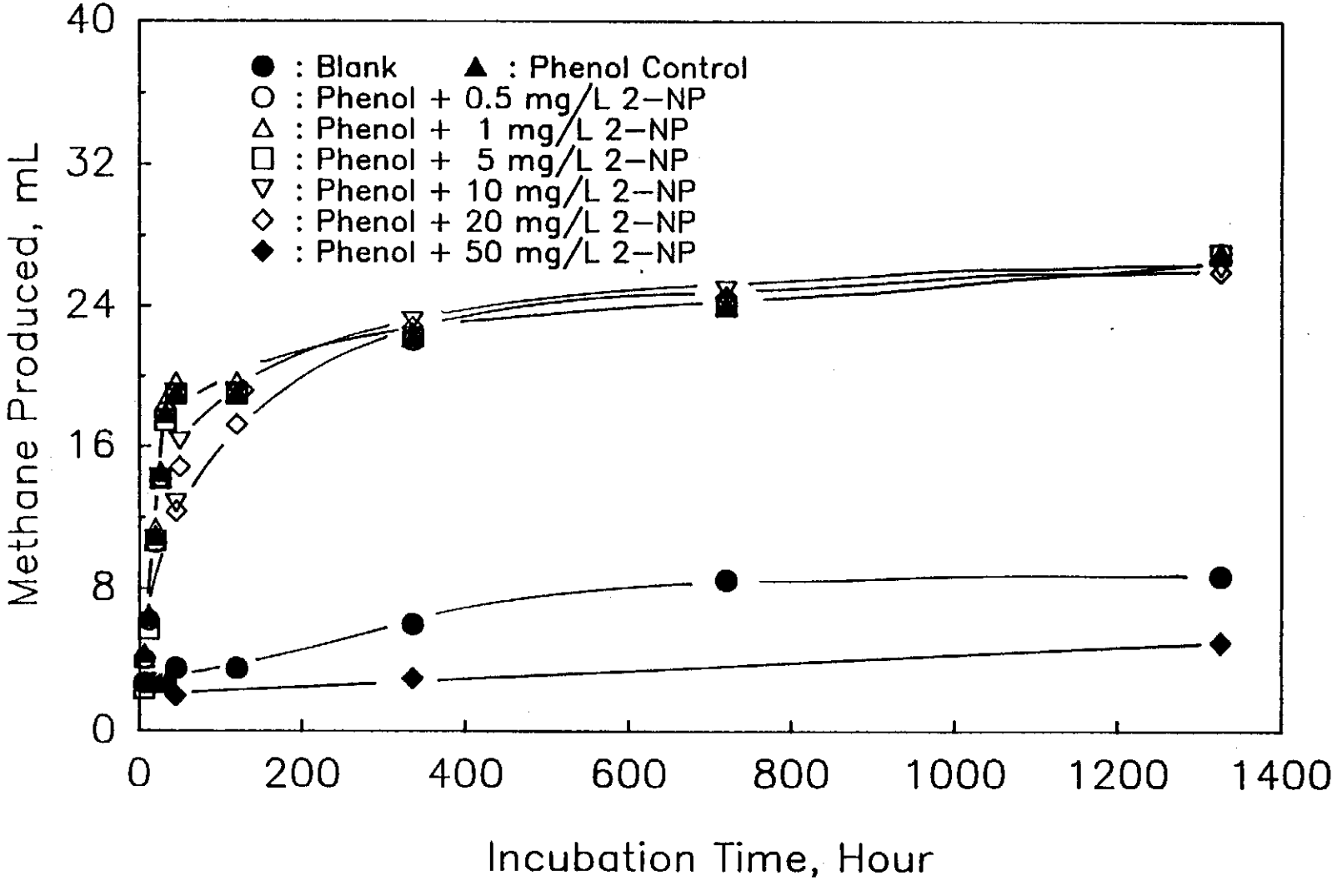

Figure 44. Methane Production from Phenol-supplemented Culture in the Presence of 2-NP

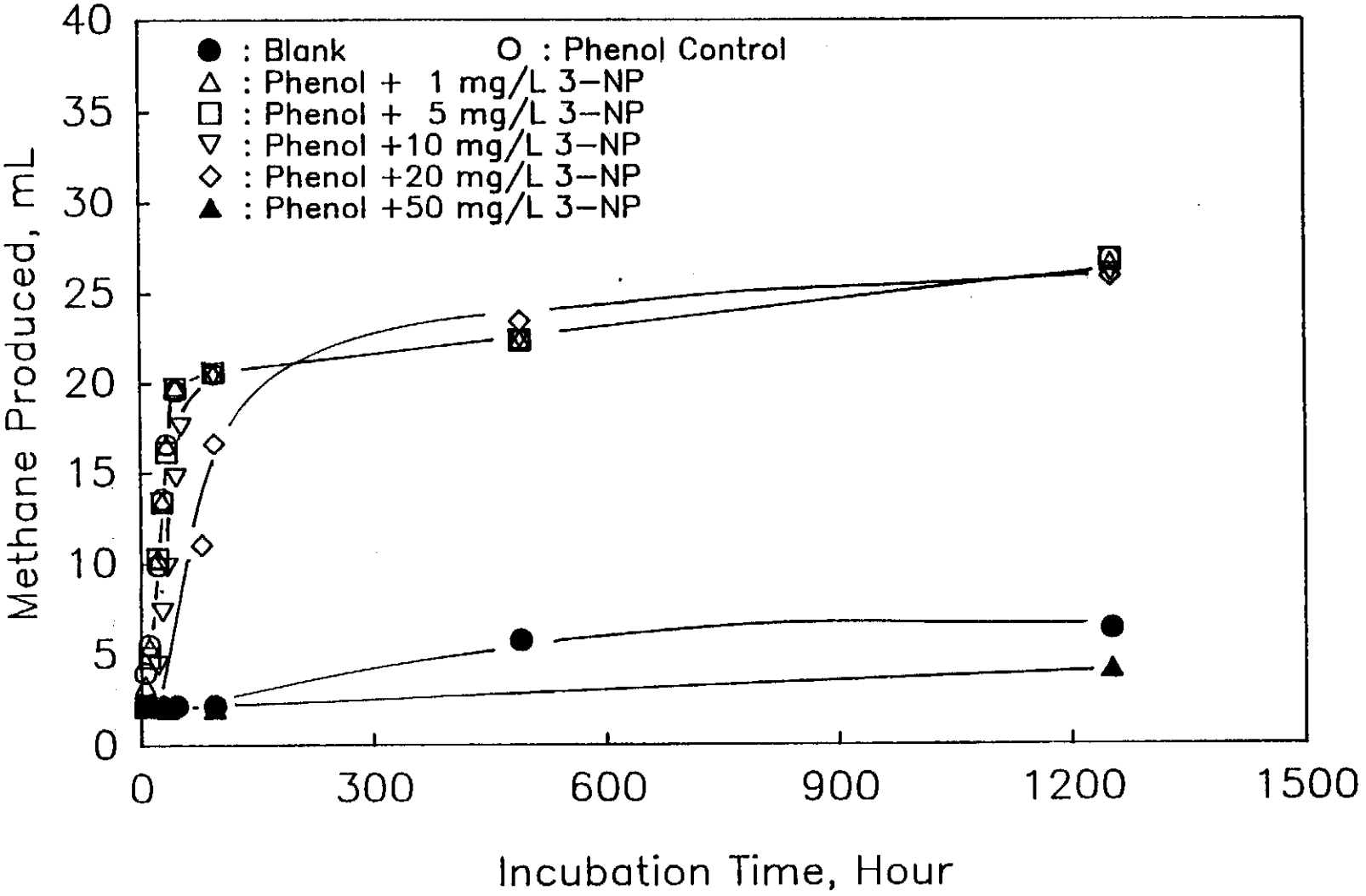

Figure 45. Methane Production from Phenol-supplemented Culture in the Presence of 3-NP

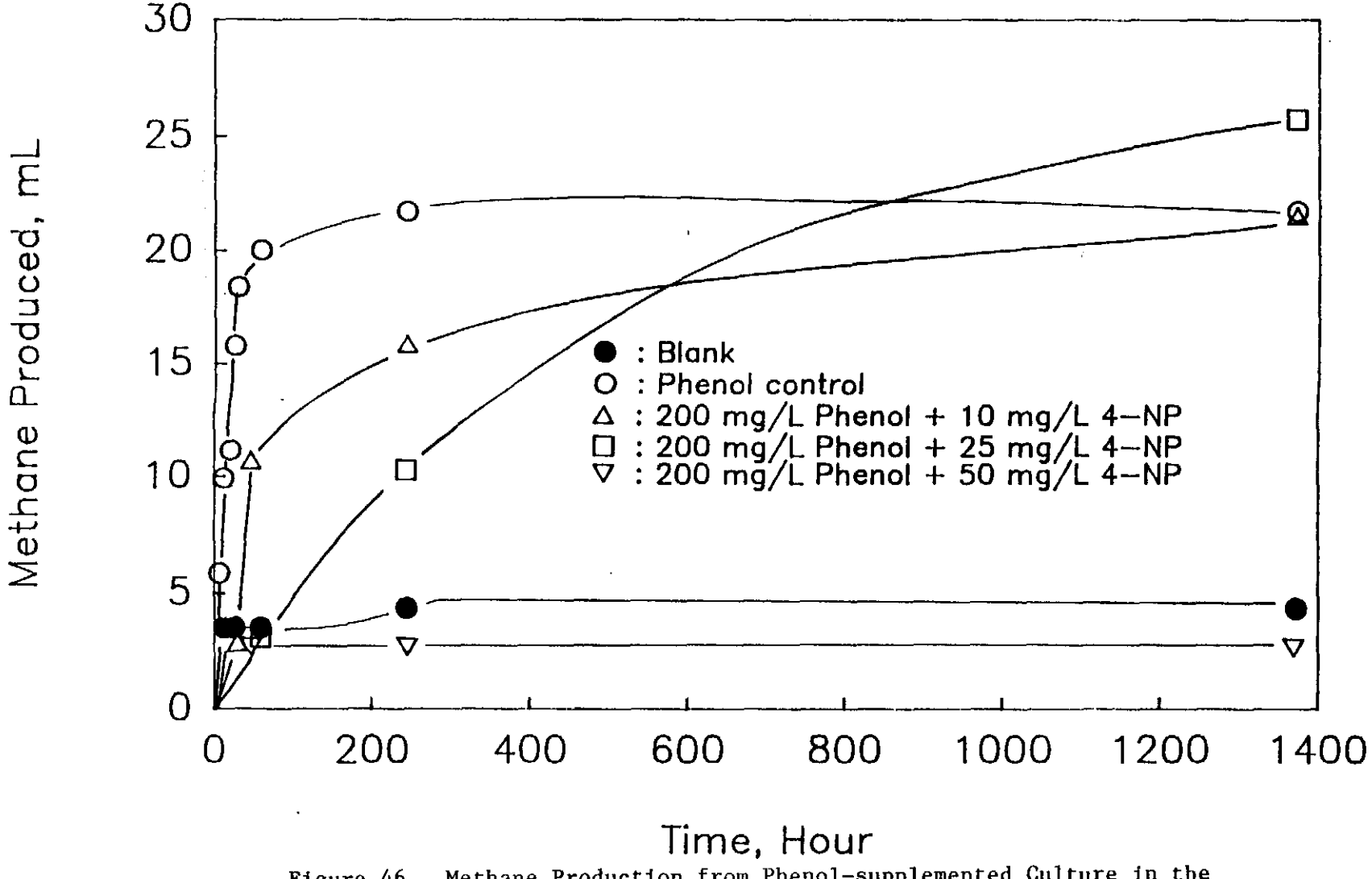

Figure 46. Methane Production from Phenol-supplemented Culture in the Presence of 4-NP

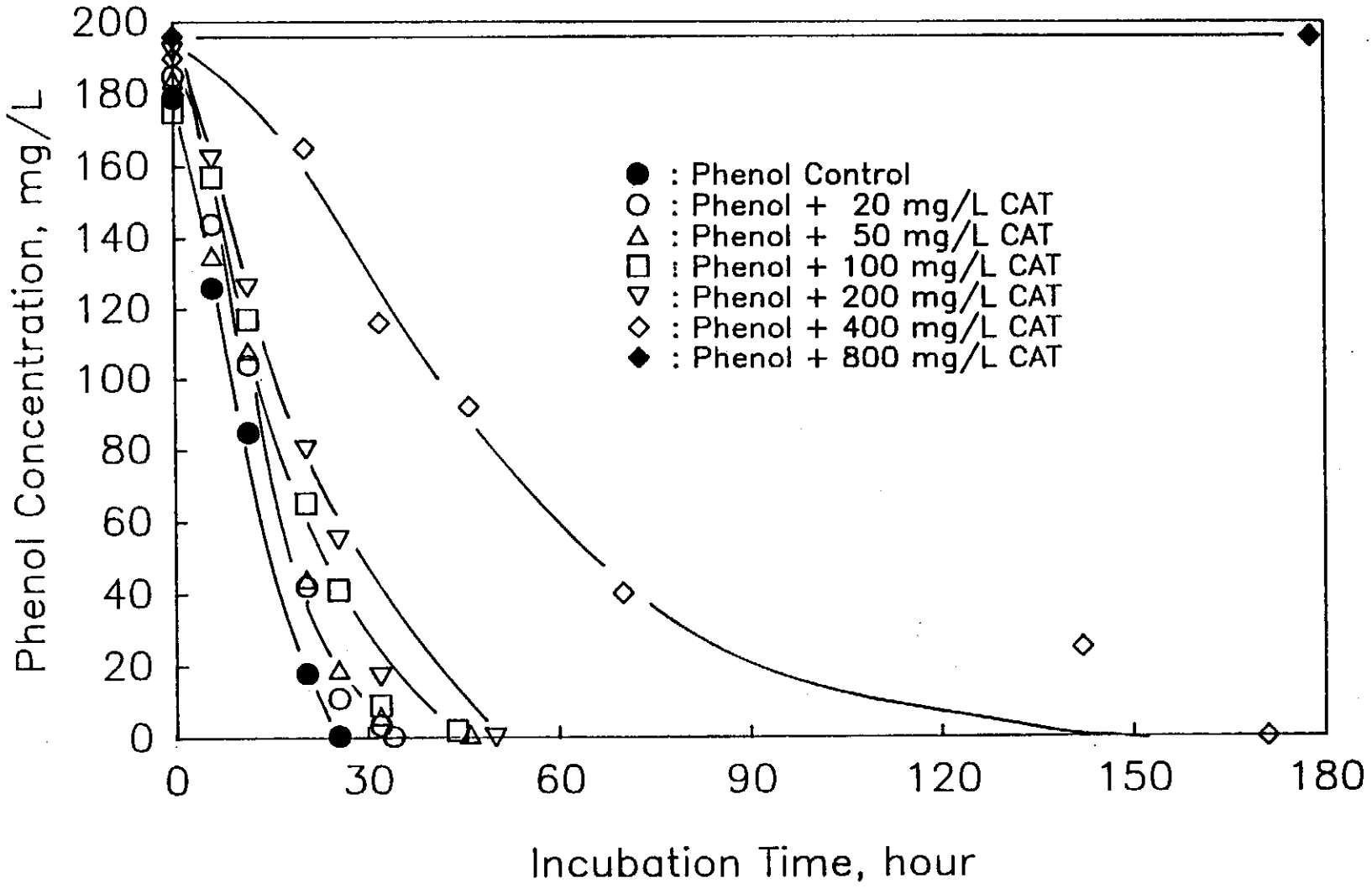

Figure 47. Effect of Catechol Concentration on Anaerobic Degradation of Phenol

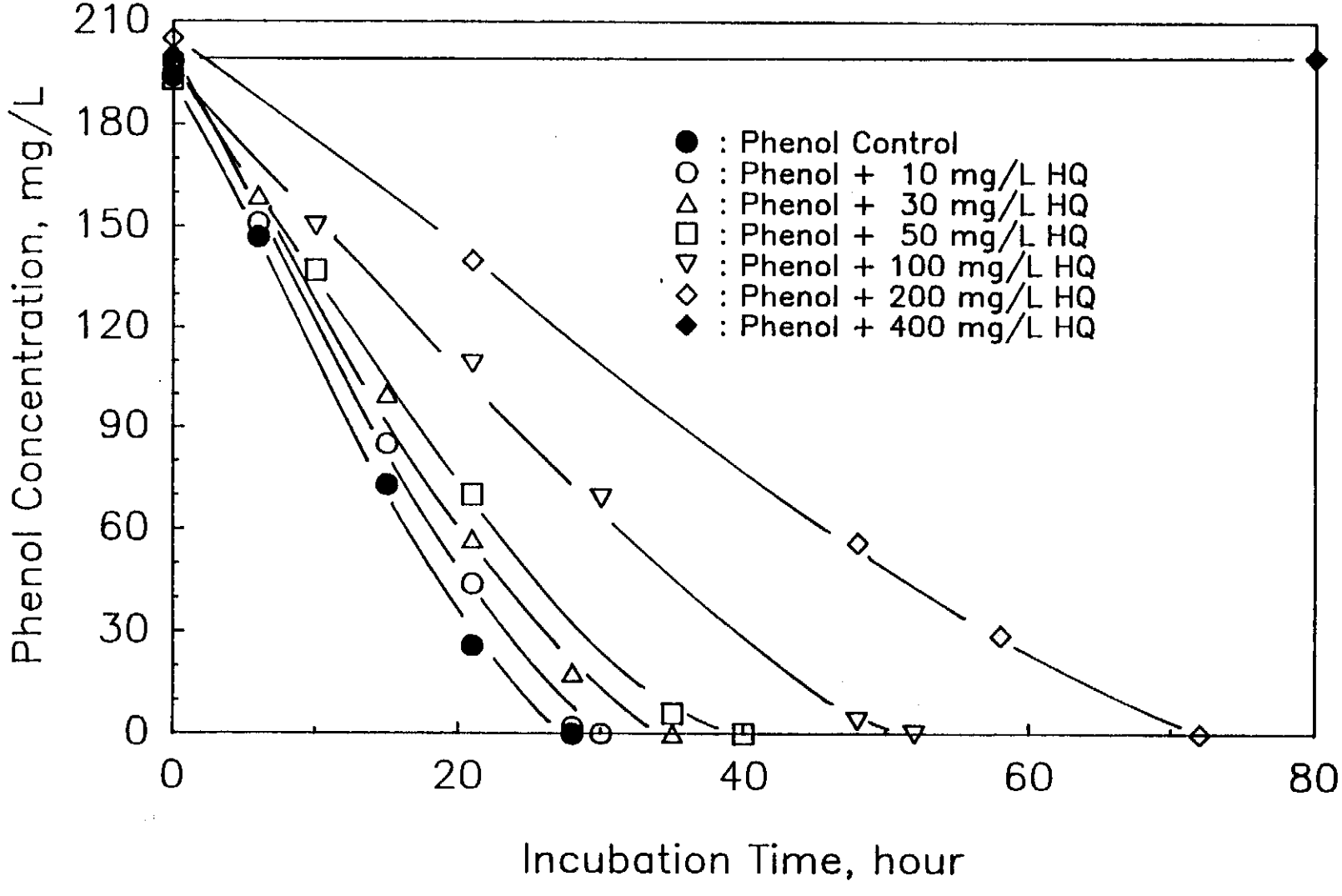

Figure 48. Effect of Hydroquinone Concentration on Anaerobic Degradation of Phenol

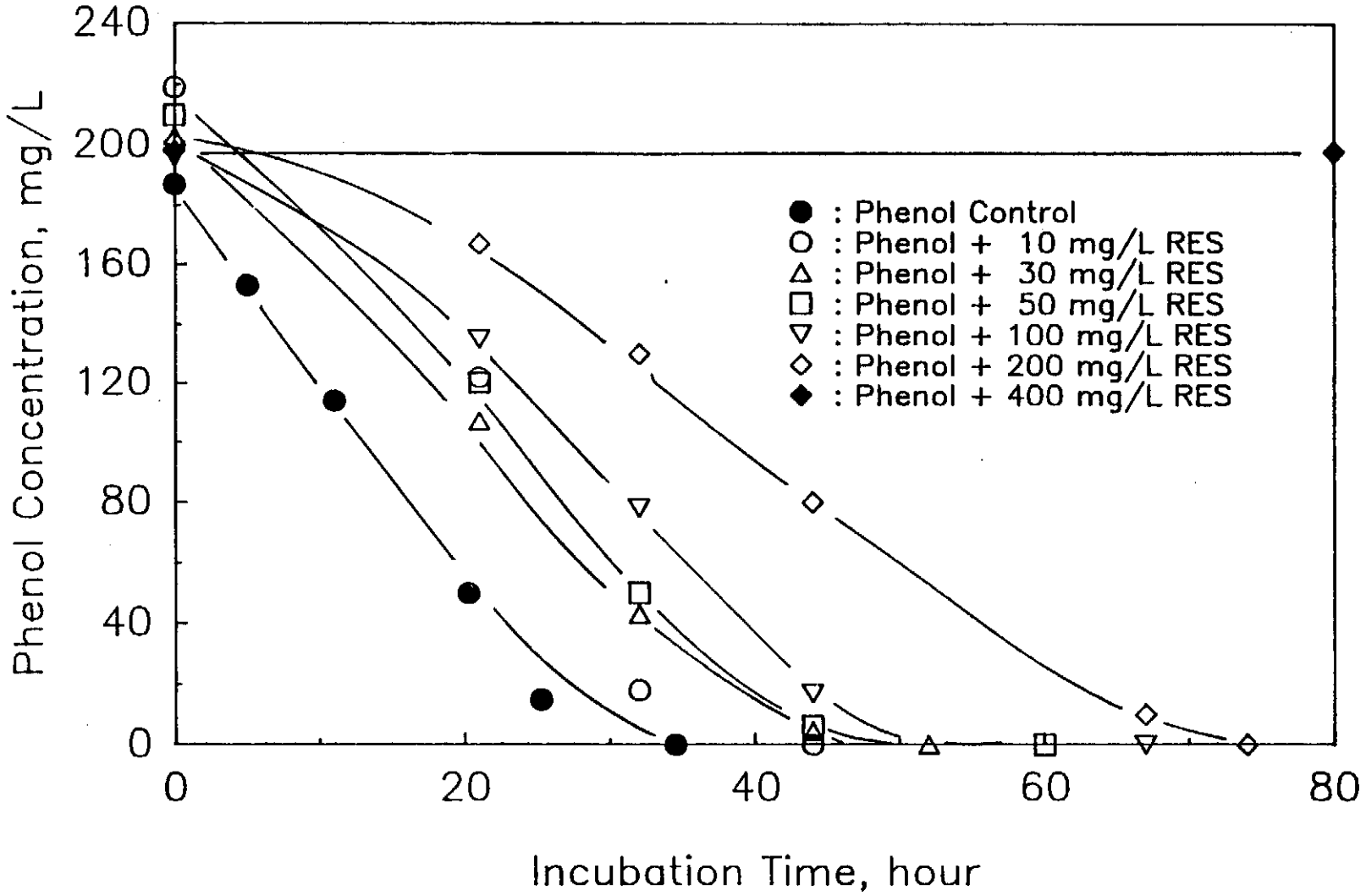

Figure 49. Effect of Resorcinol Concentration on Anaerobic Degradation of Phenol

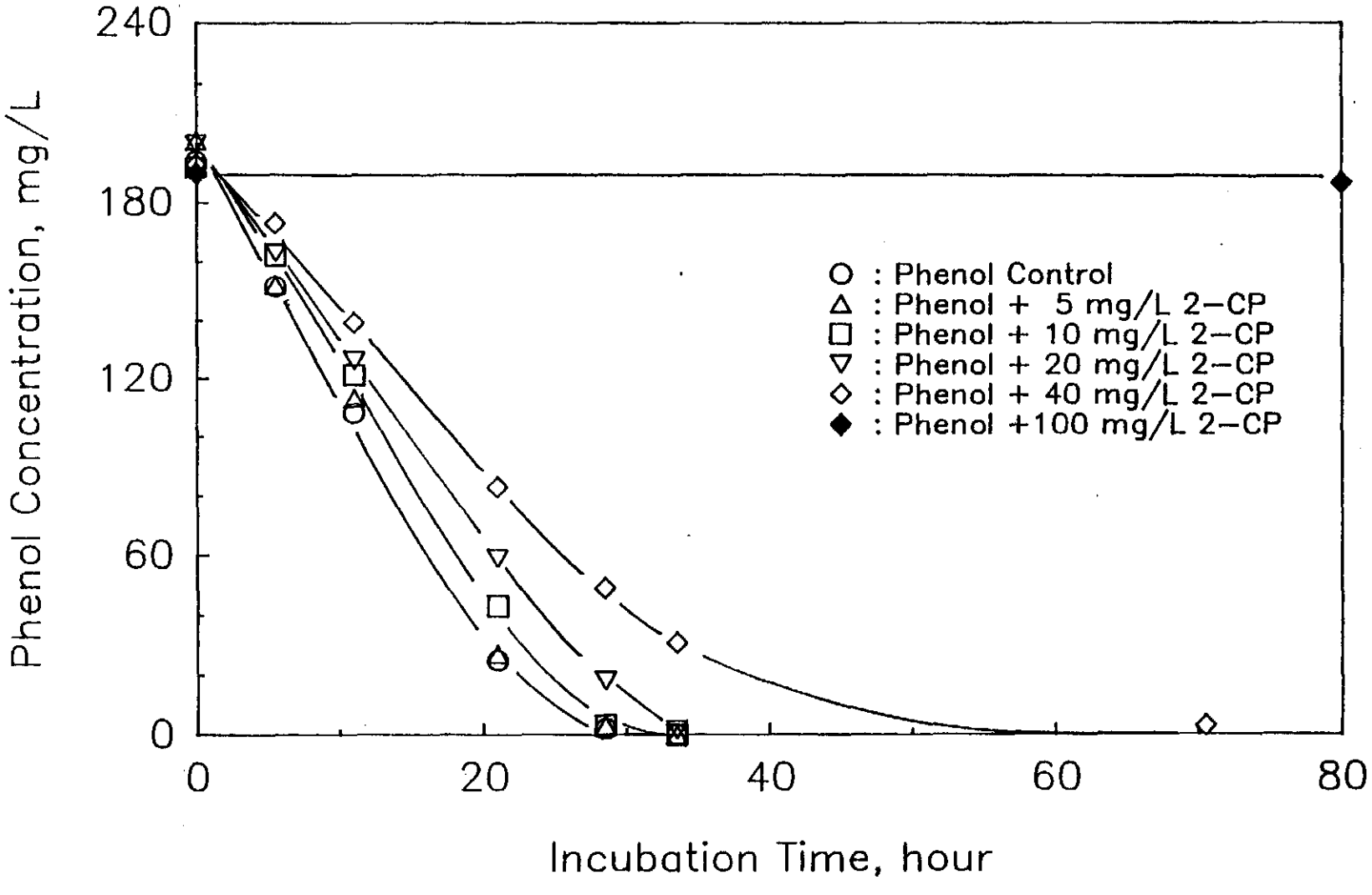

Figure 50. Effect of 2-CP Concentration on Anaerobic Degradation of Phenol

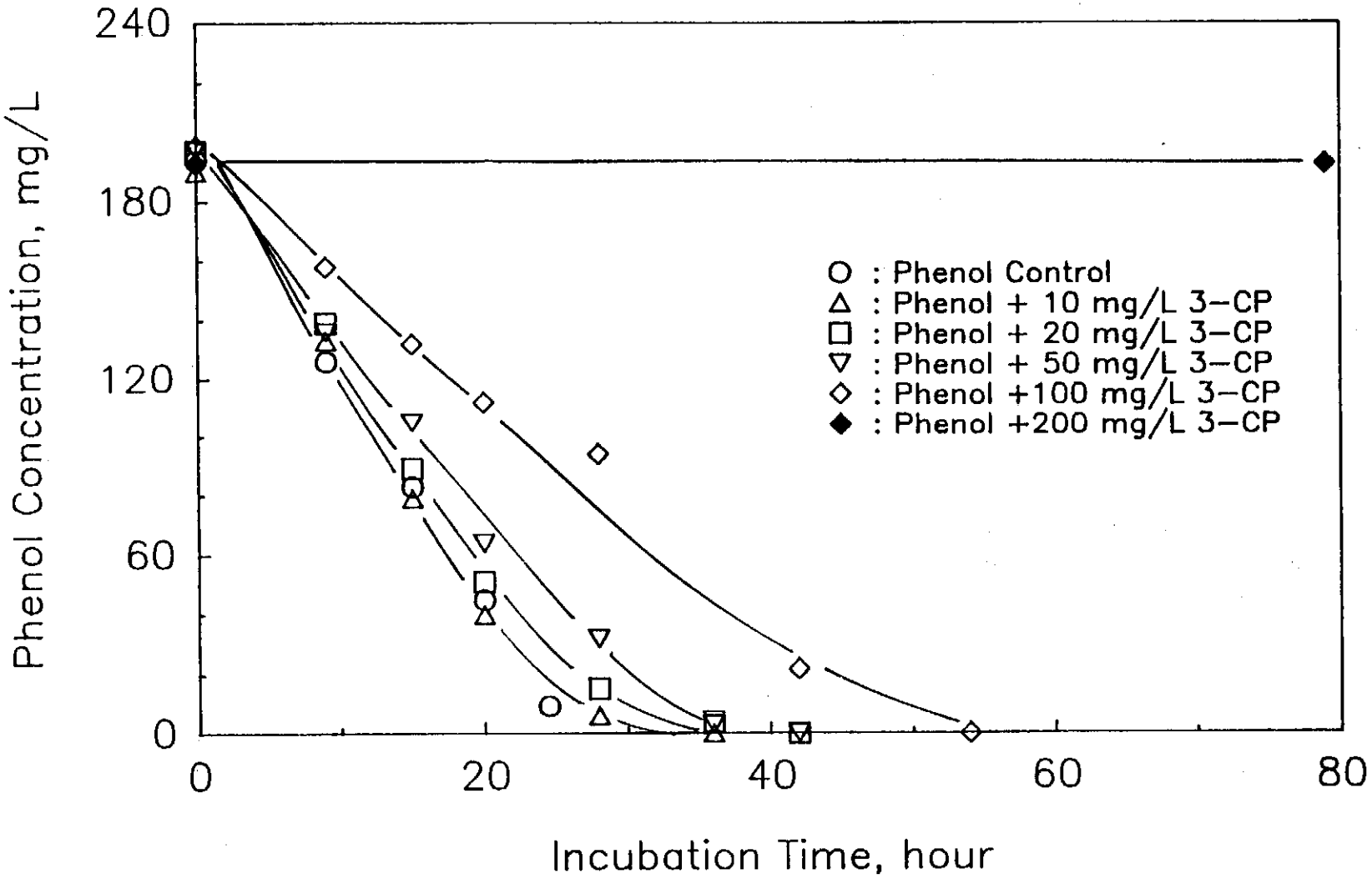

Figure 51. Effect of 3-CP Concentration on Anaerobic Degradation of Phenol

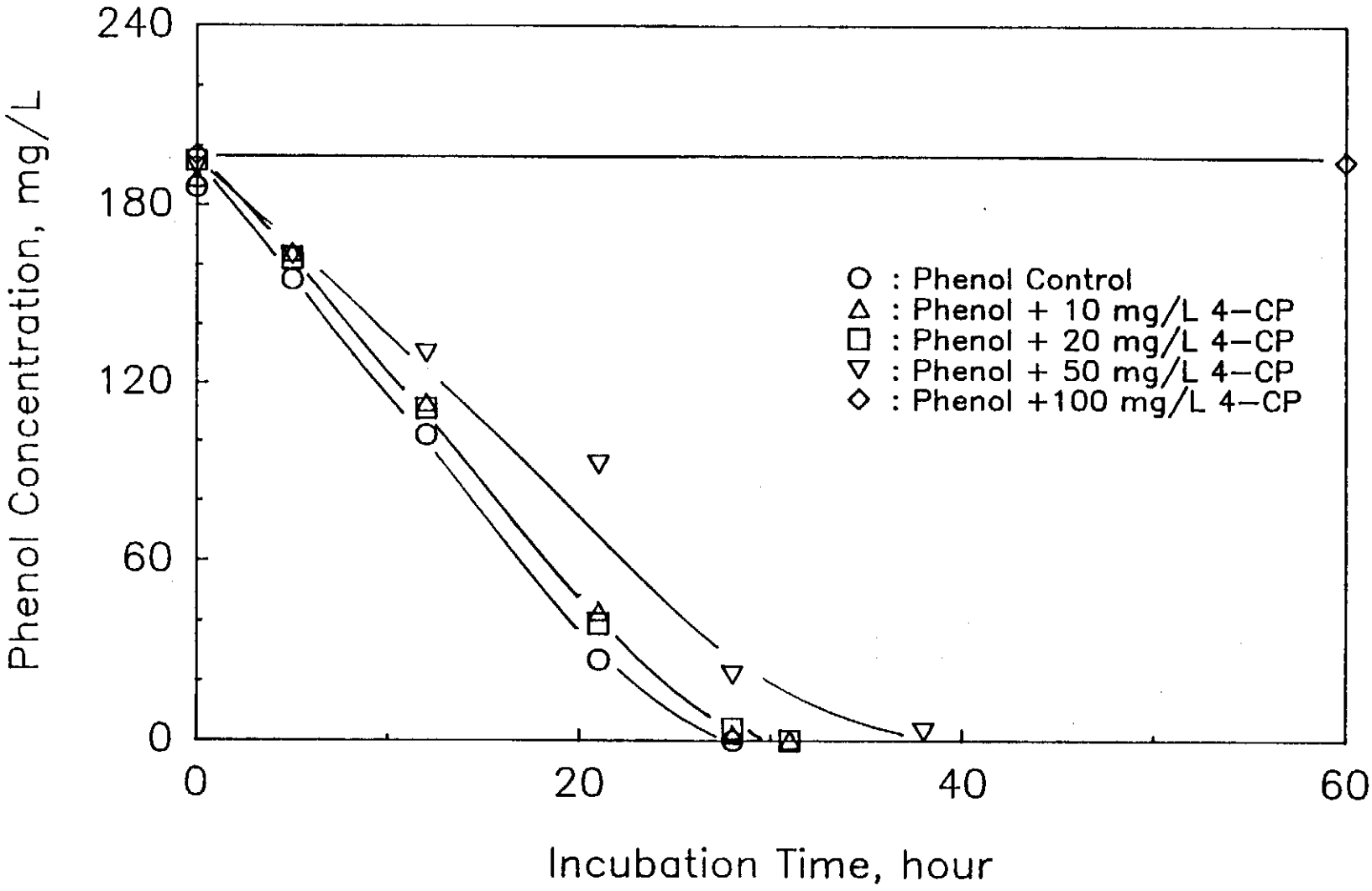

Figure 52. Effect of 4-CP Concentration on Anaerobic Degradation of Phenol

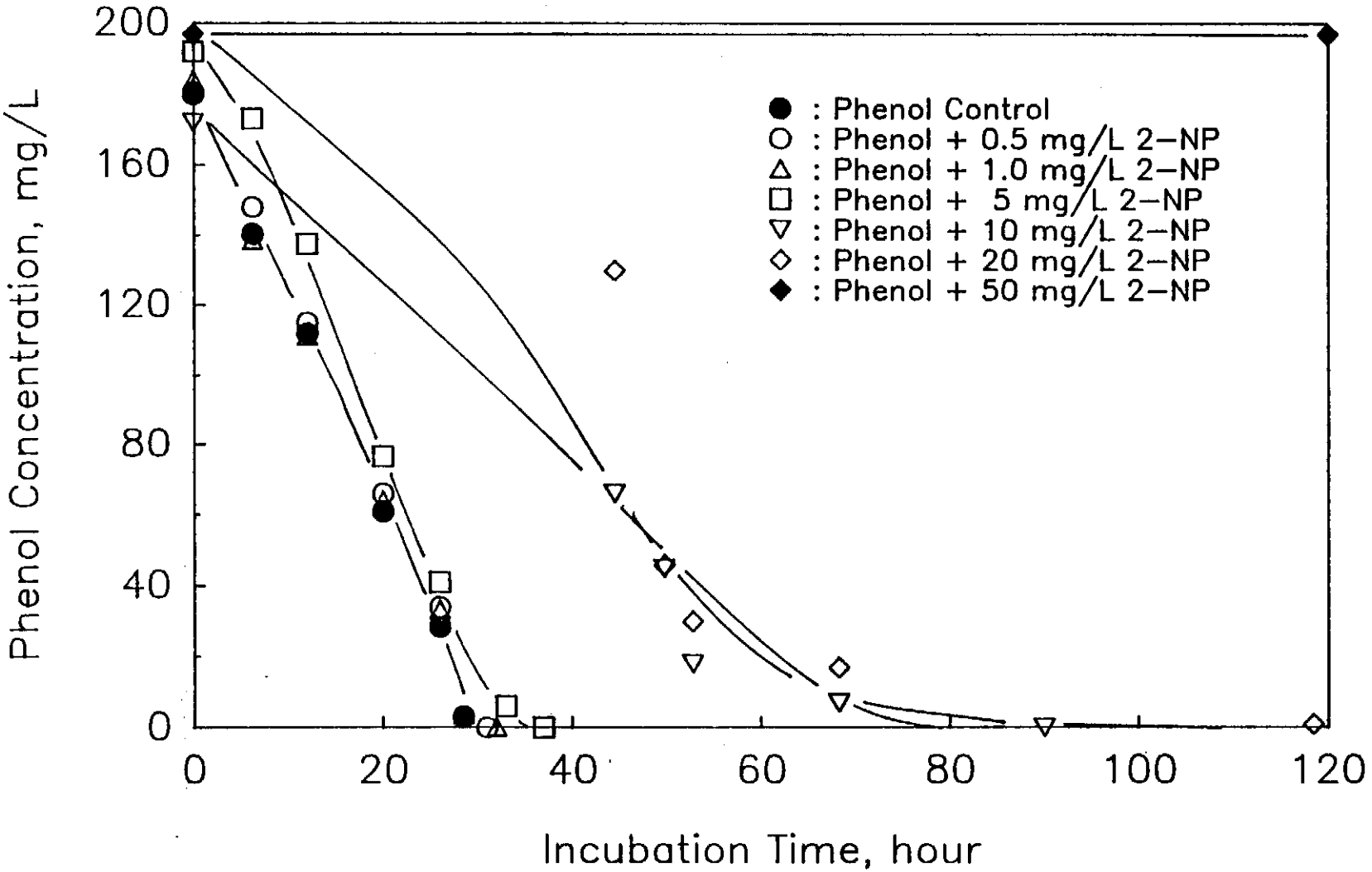

Figure 53. Effect of 2-NP Concentration on Anaerobic Degradation of Phenol

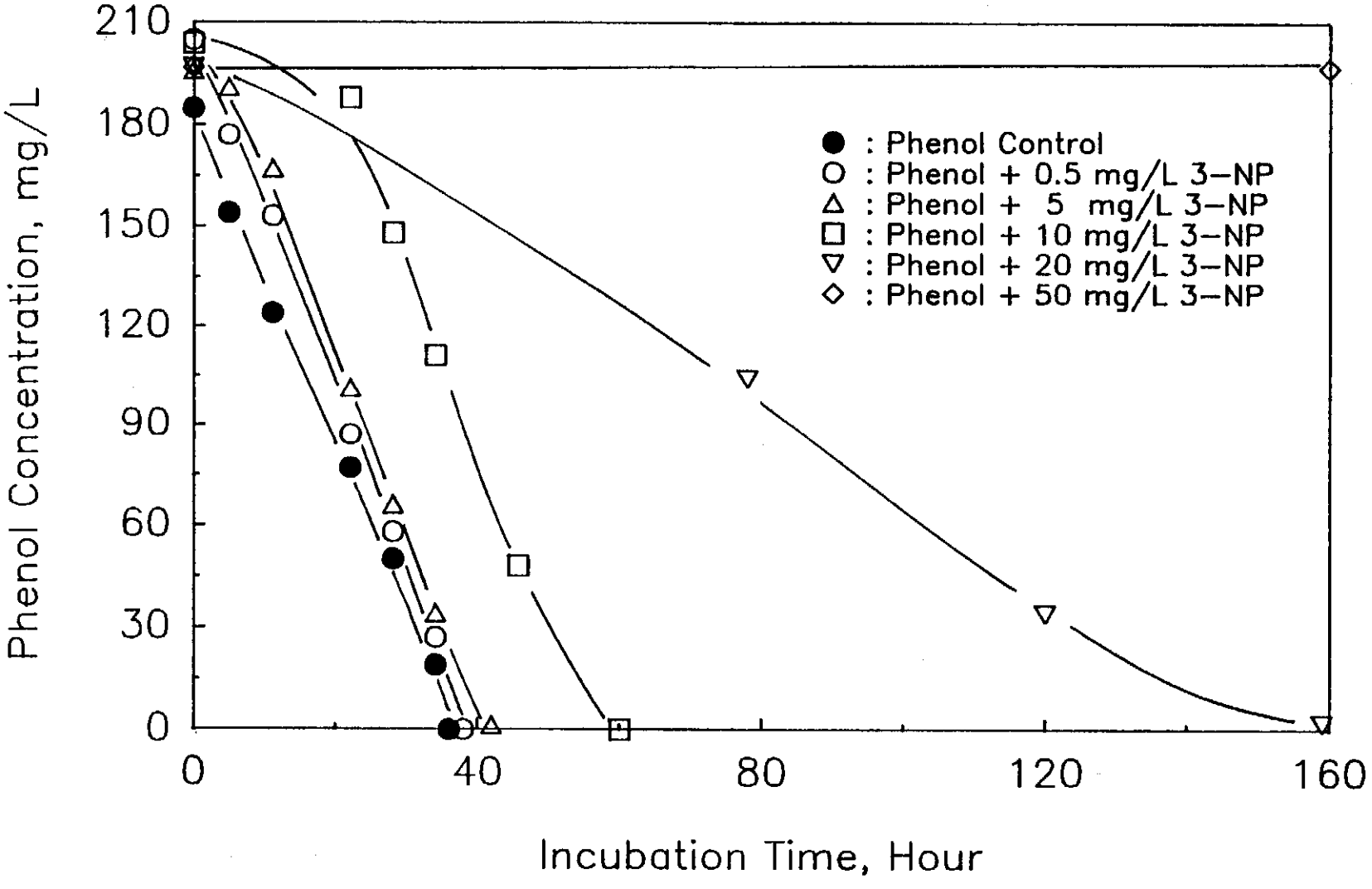

Figure 54. Effect of 3-NP Concentration on Anaerobic Degradation of Phenol

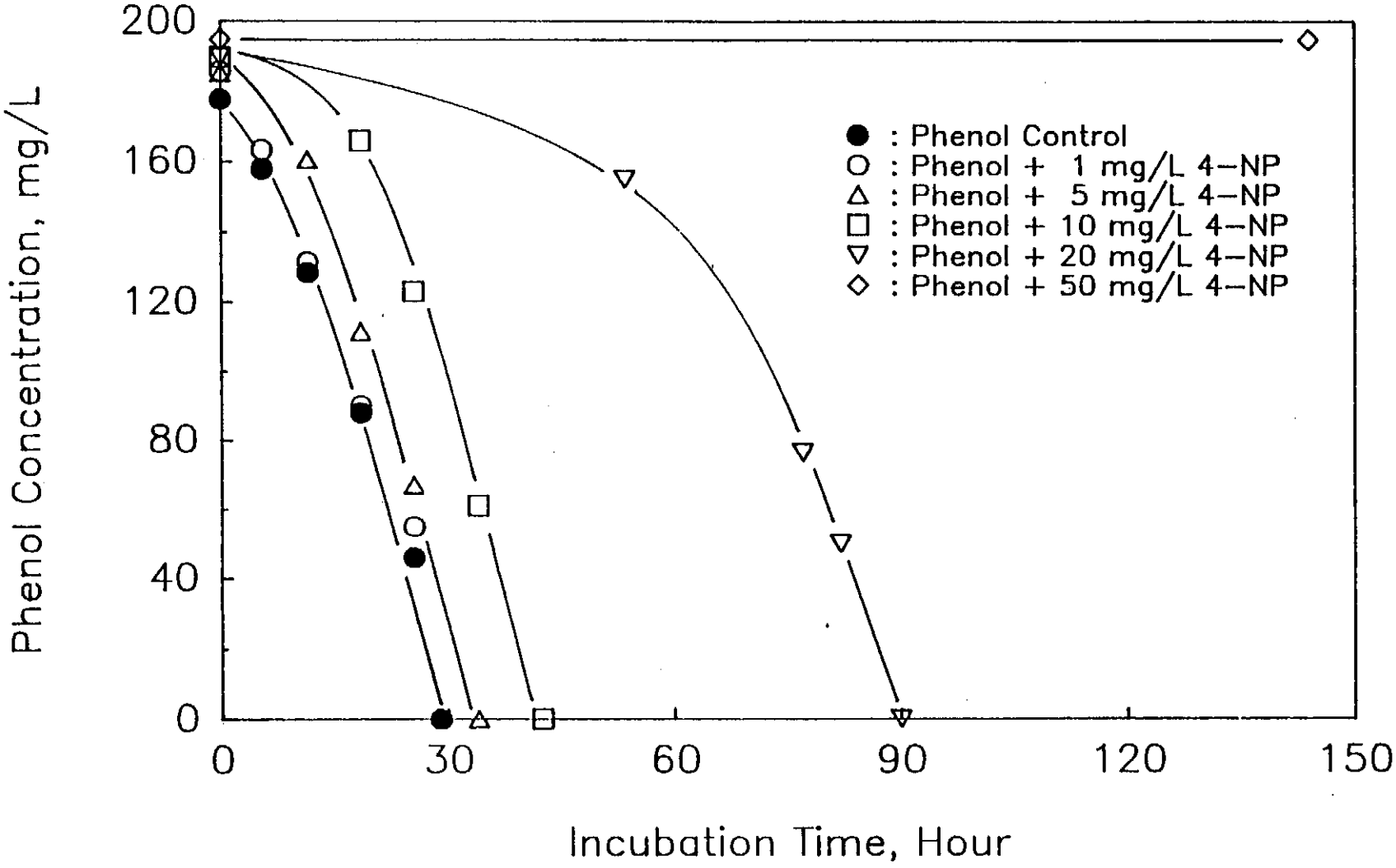

Figure 55. Effect of 4-CP Concentration on Anaerobic Degradation of Phenol

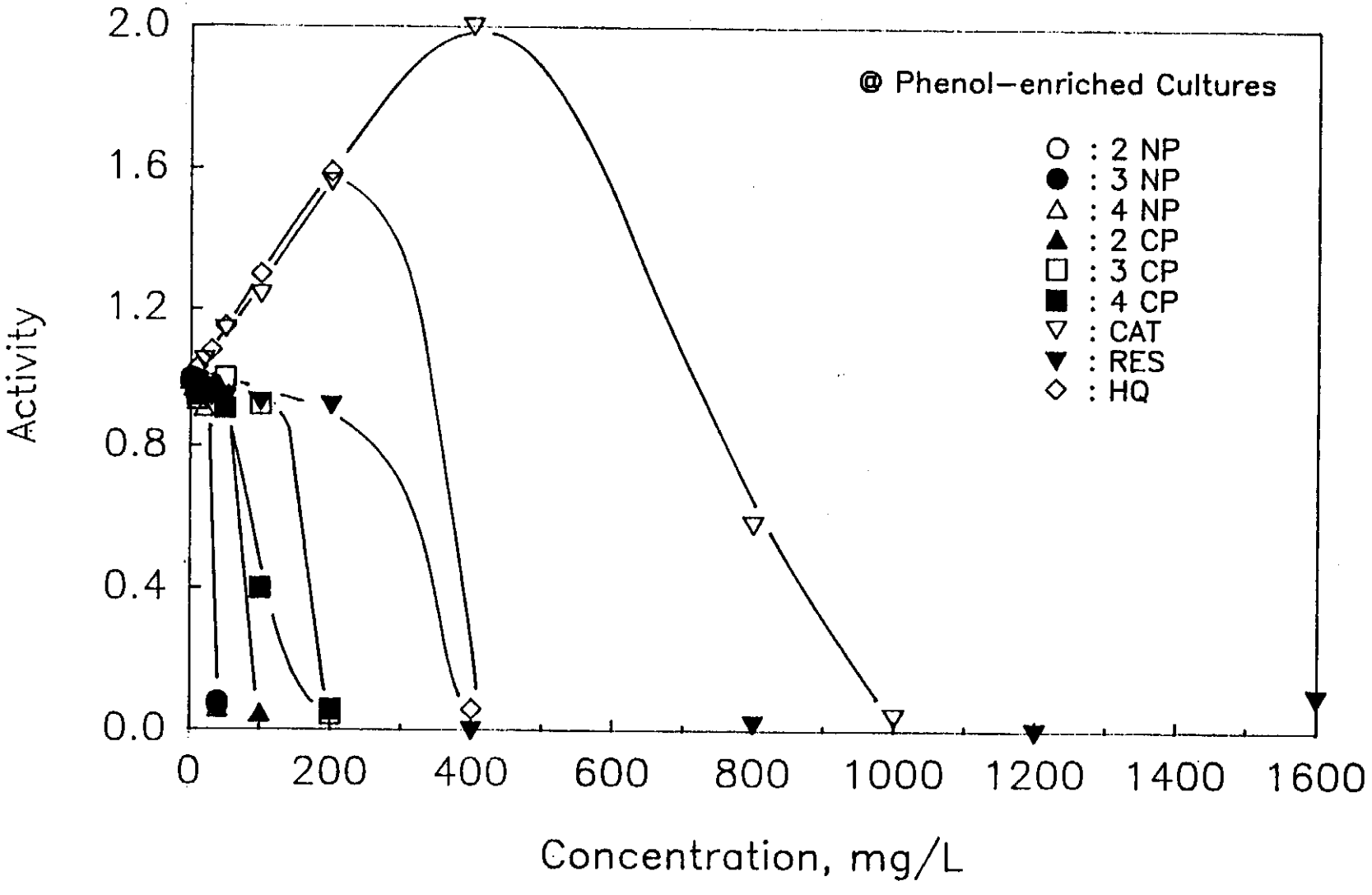

Figure 56. Effect of Phenolic Concentration on Methanogenic Activity in the Phenol-enriched Culture

Table 14 Toxicity Levels of Phenols for Phenol-enriched Culture

Compound	Concentration corresponding to 50% activity reduction, mg/ℓ	Concentration corresponding to no activity, mg/ℓ
Catechol	840	1000
Hydroquinone	350	400
Resorcinol	340	400
2-CP	70	100
3-CP	160	200
4-CP	100	200
2-NP	20	50
3-NP	20	50
4-NP	20	50

thresholds may indicate culture acclimation to catechol.

The growth of bacterial population was monitored throughout the entire assay in addition to the fate of phenolic compounds. The fate of the substituted phenols as shown in Figures 57-91 was not significantly affected by the presence of biodegradable phenol as compared to results obtained with these individual compounds (Figures 11 and 12 and Table 7). In addition, the biomass remained rather constant in spite of inhibition (Figures 92-103).

Data Analysis

Substrate inhibition. Batch test data from phenol and catechol were analyzed, respectively, using the Haldane kinetic equation for substrate utilization:

$$-\frac{dS}{dt} = \frac{k\ X\ S}{K_s+S+S^2/K_I} \qquad (1)$$

where X is the cell mass concentration, S is the substrate concentration, K_s is the Monod half-velocity coefficient, k is the maximum substrate utilization rate, K_I is the Haldane inhibition constant. Because the biomass concentration remained rather constant throughout the incubation (Figures 92-103), the biomass X was assumed to be constant. Consequently, biomass yield and decay were not considered by this approach.

Equation (1) was integrated and rearranged in order to obtain partial derivatives of the model with respect to each parameter:

$$\frac{\partial t}{\partial K_s} = \frac{1}{kX_o} \ln (S_o/S) \qquad (2)$$

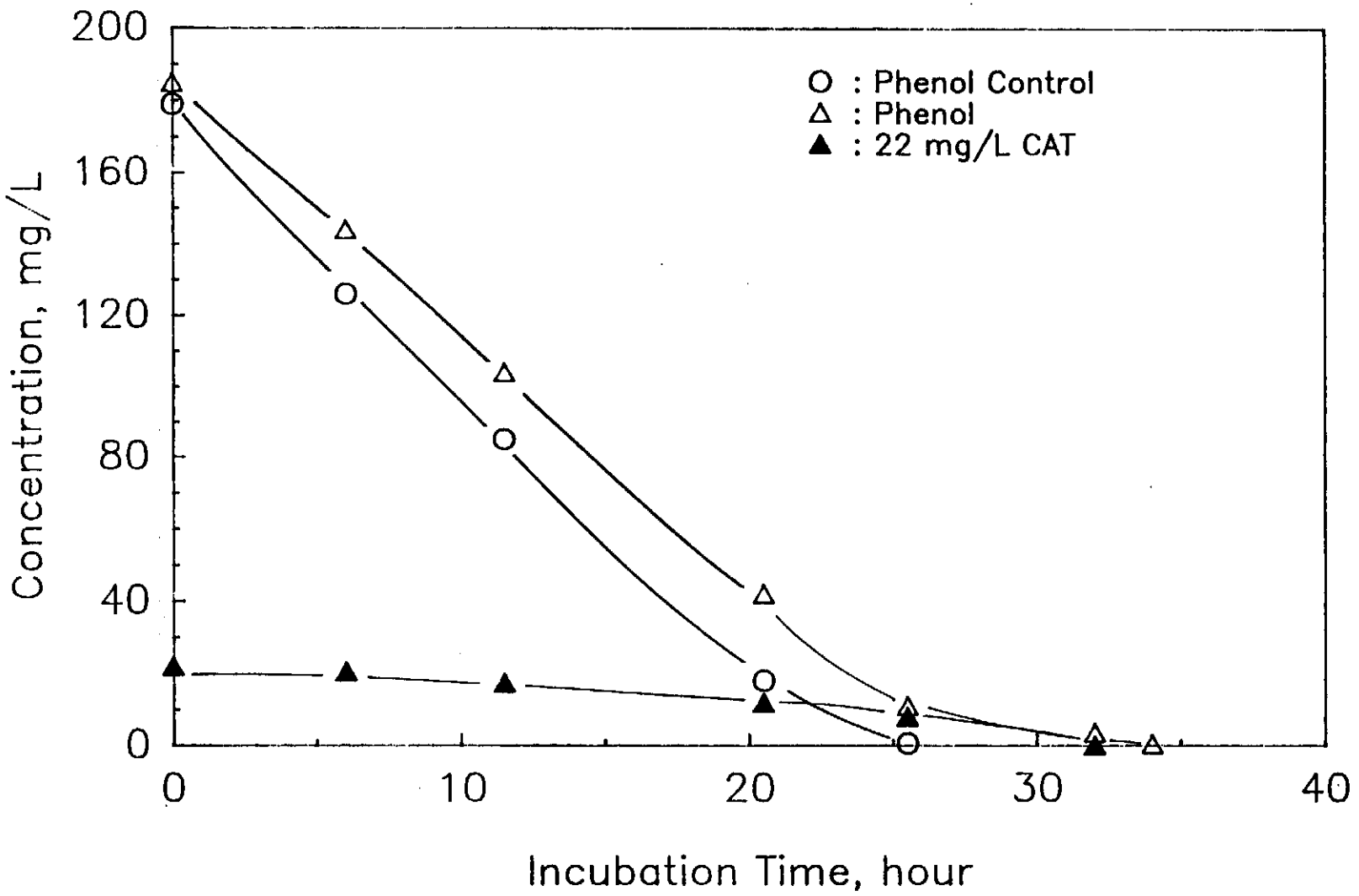

Figure 57. Fate of Catechol in the Phenol-supplemented Culture: Initial Concentration of 22 mg/L

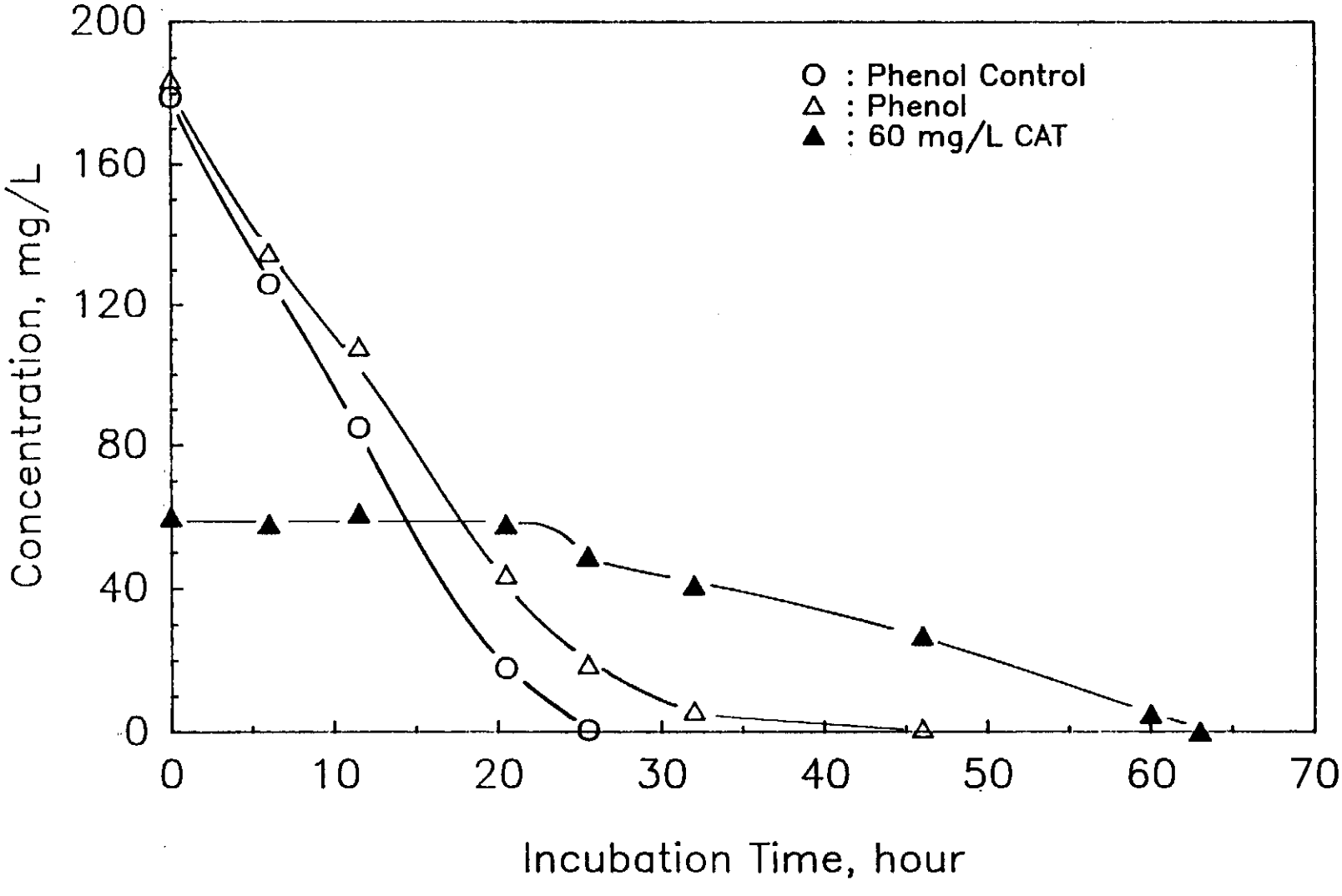

Figure 58. Fate of Catechol in the Phenol-supplemented Culture: Initial Concentration of 60 mg/L

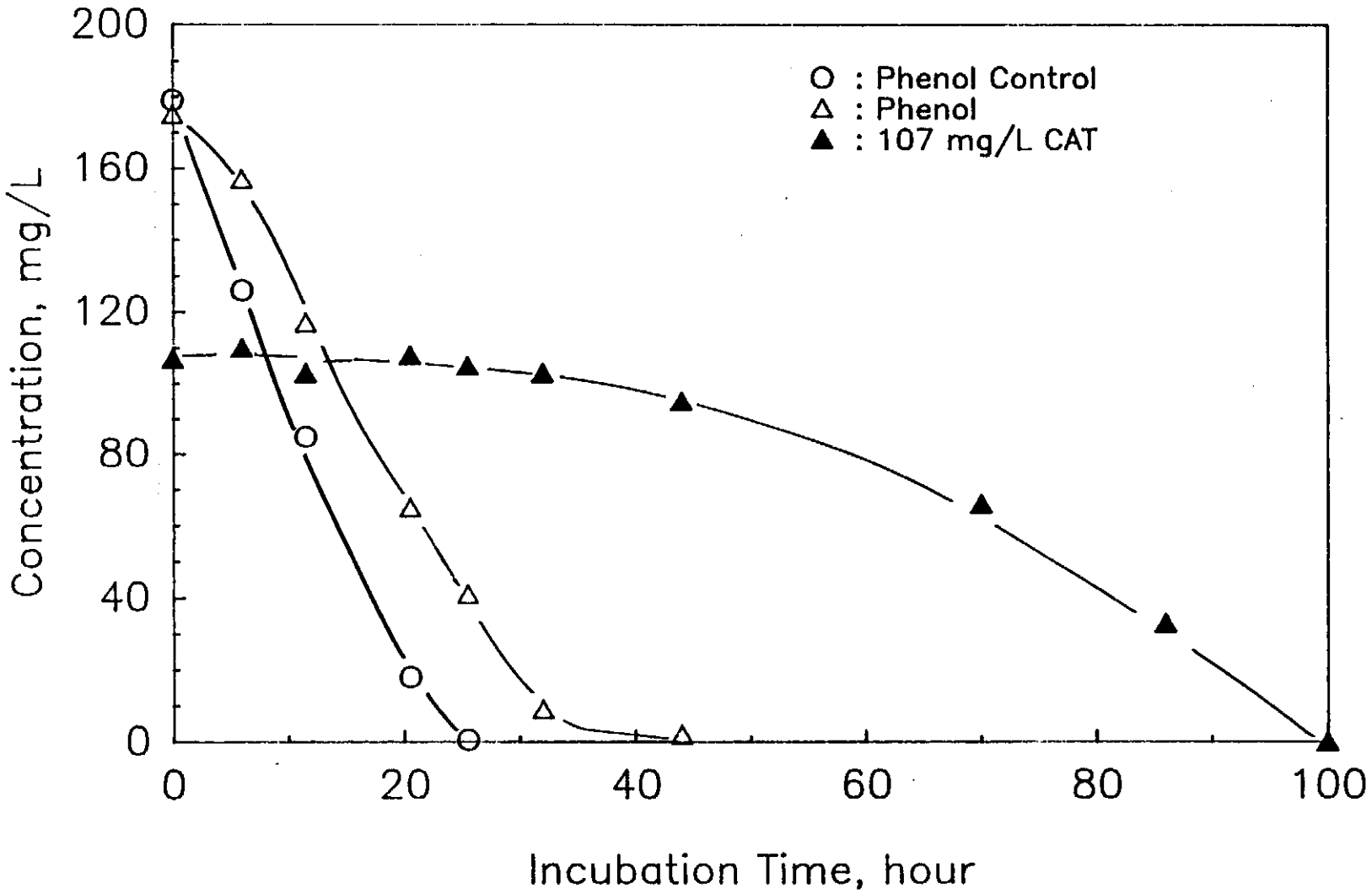

Figure 59. Fate of Catechol in the Phenol-supplemented Culture: Initial Concentration of 107 mg/L

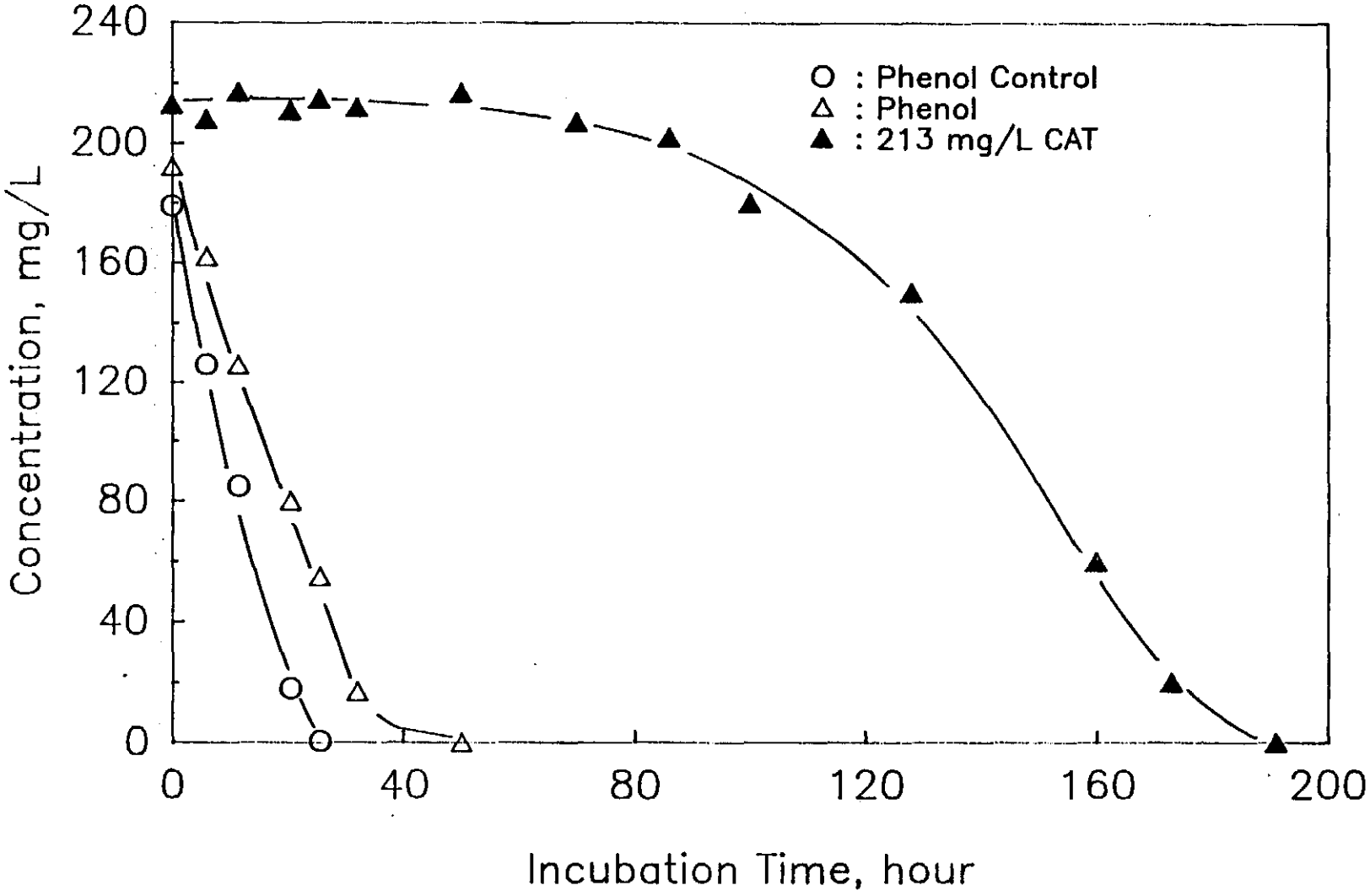

Figure 60. Fate of Catechol in the Phenol-supplemented Culture: Initial Concentration of 213 mg/L

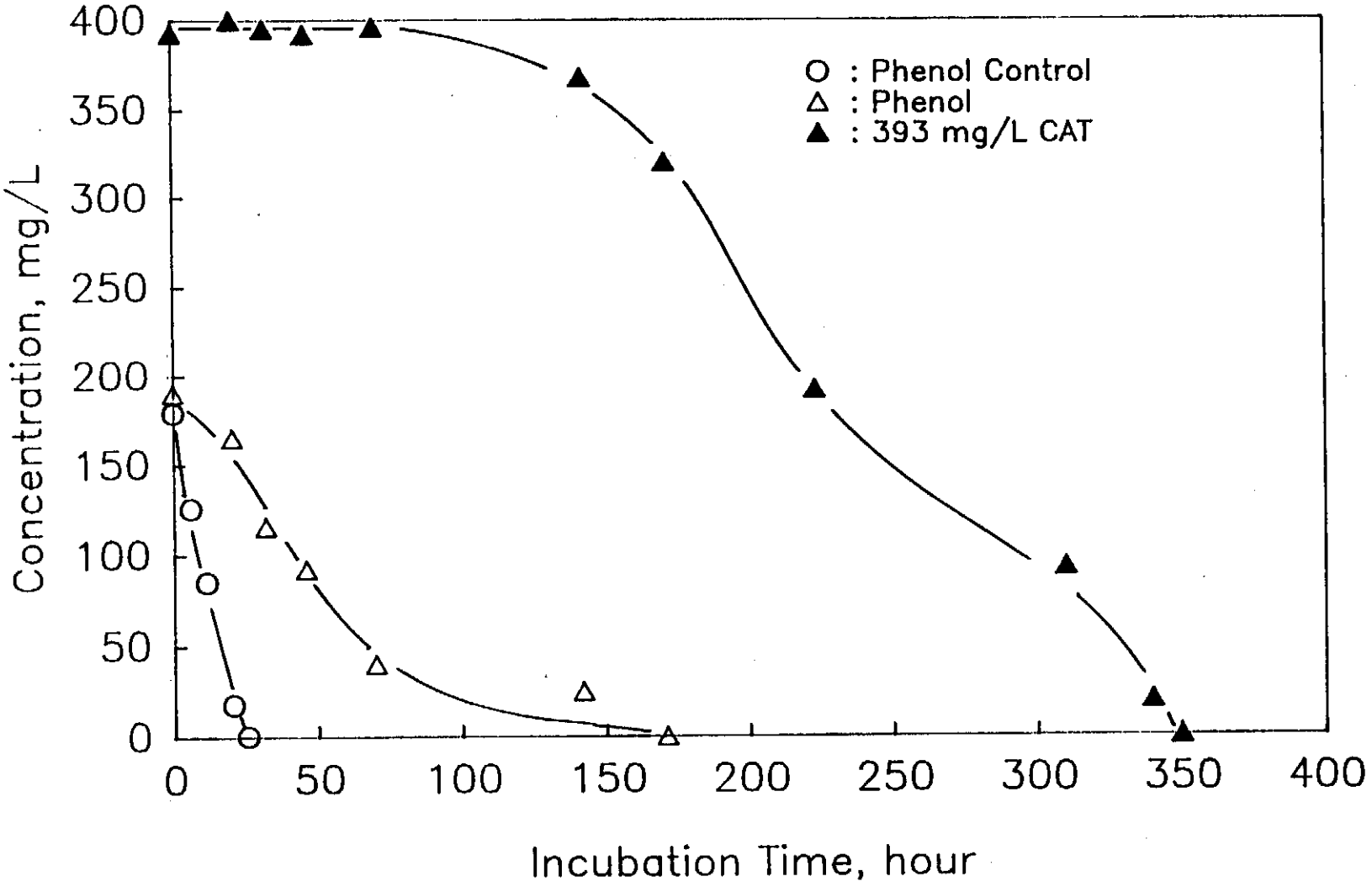

Figure 61. Fate of Catechol in the Phenol-supplemented Culture: Initial Concentration of 393 mg/L

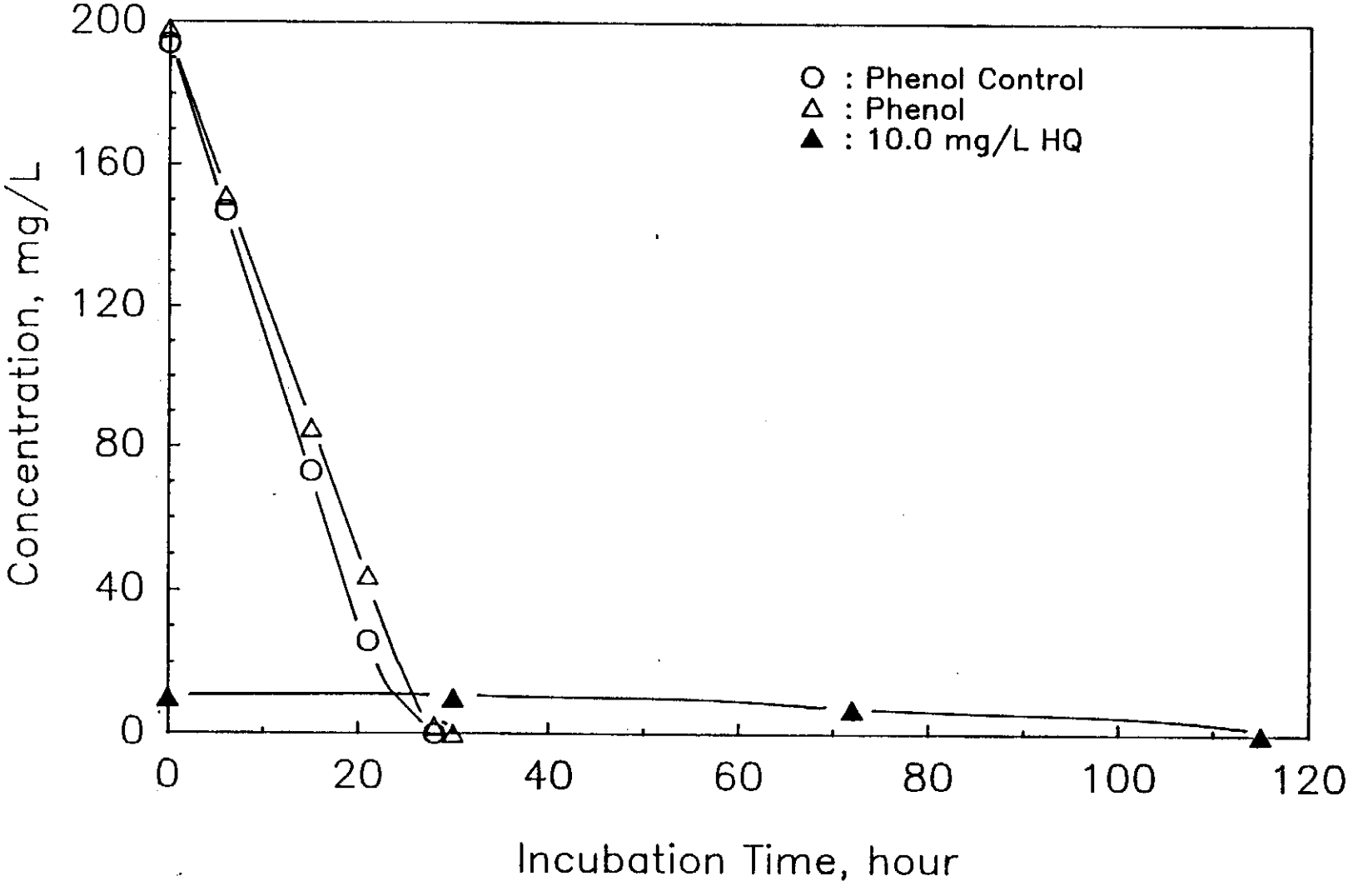

Figure 62. Fate of Hydroquinone in the Phenol-supplemented Culture: Initial Concentration of 10 mg/L

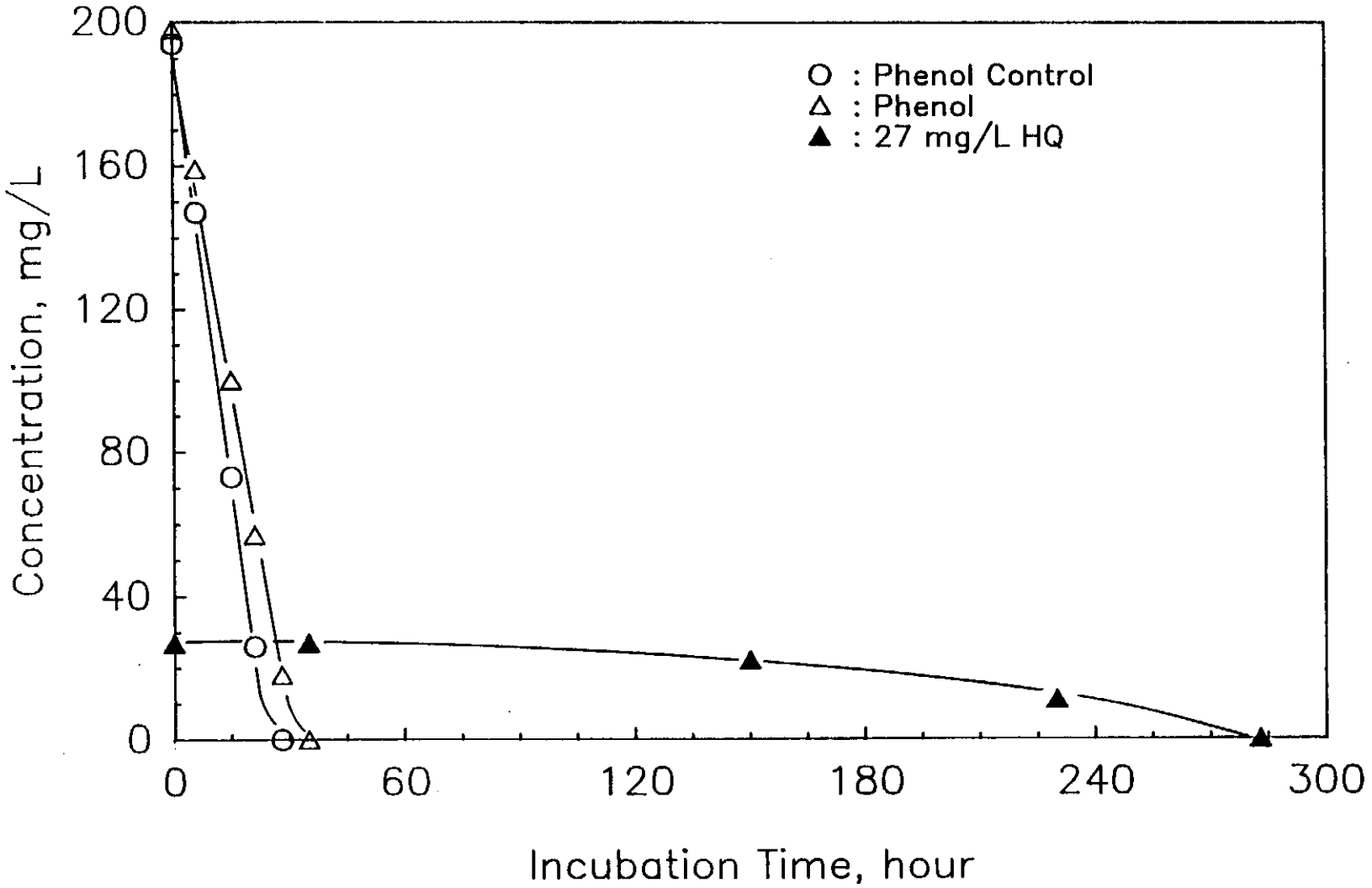

Figure 63. Fate of Hydroquinone in the Phenol-supplemented Culture: Initial Concentration of 27 mg/L

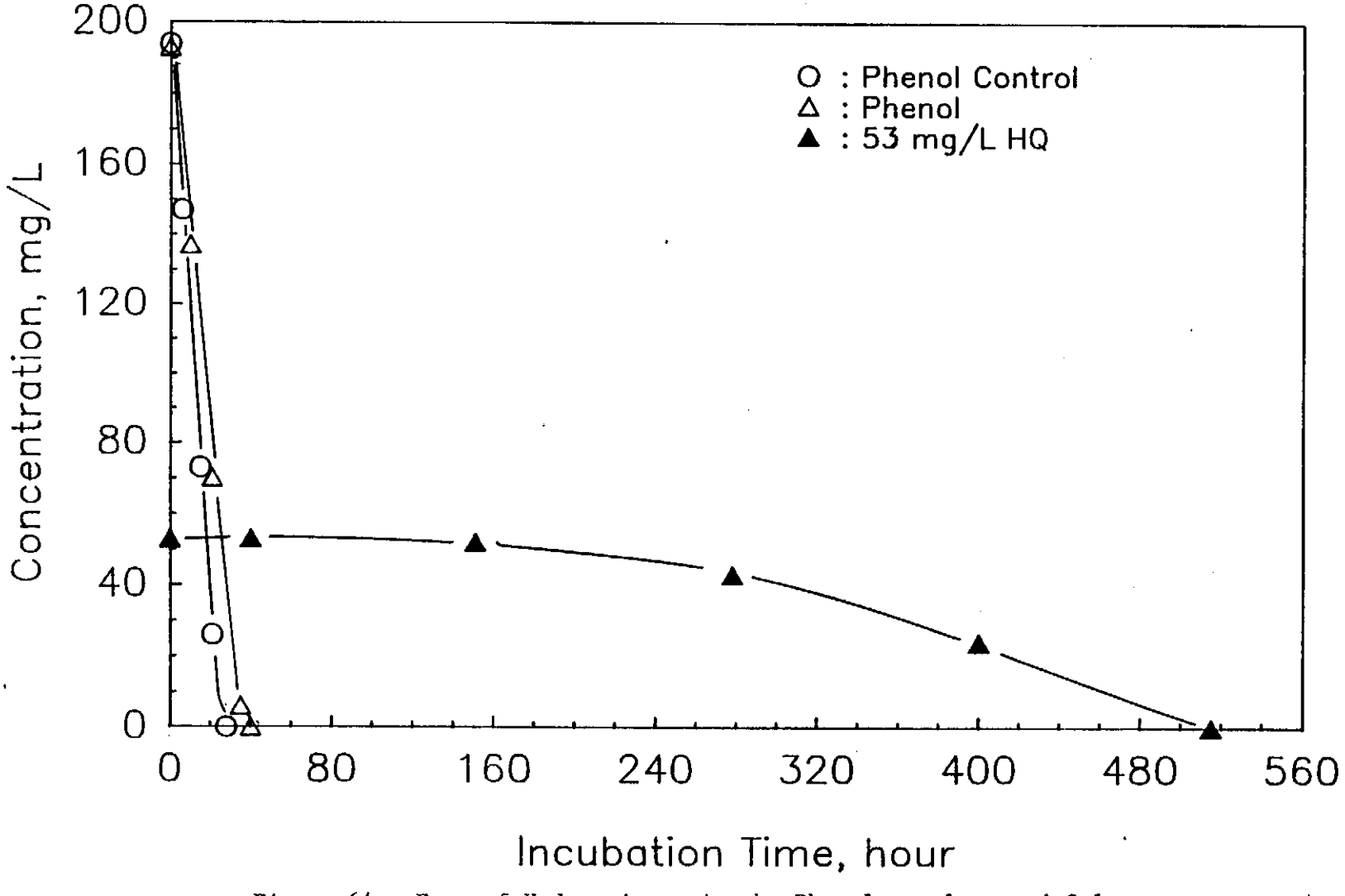

Figure 64. Fate of Hydroquinone in the Phenol-supplemented Culture: Initial Concentration of 53 mg/L

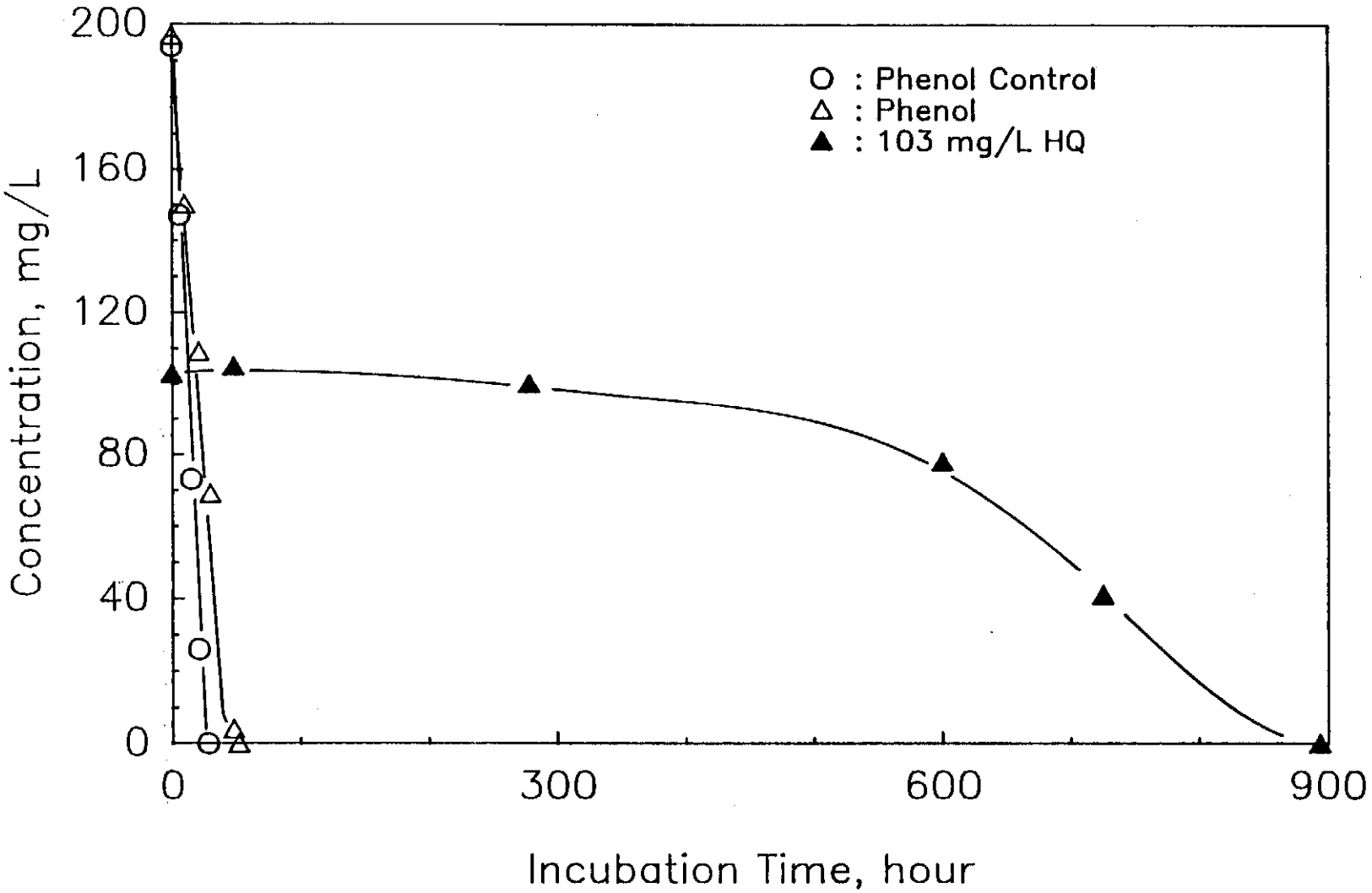

Figure 65. Fate of Hydroquinone in the Phenol-supplemented Culture: Initial Concentration of 103 mg/L

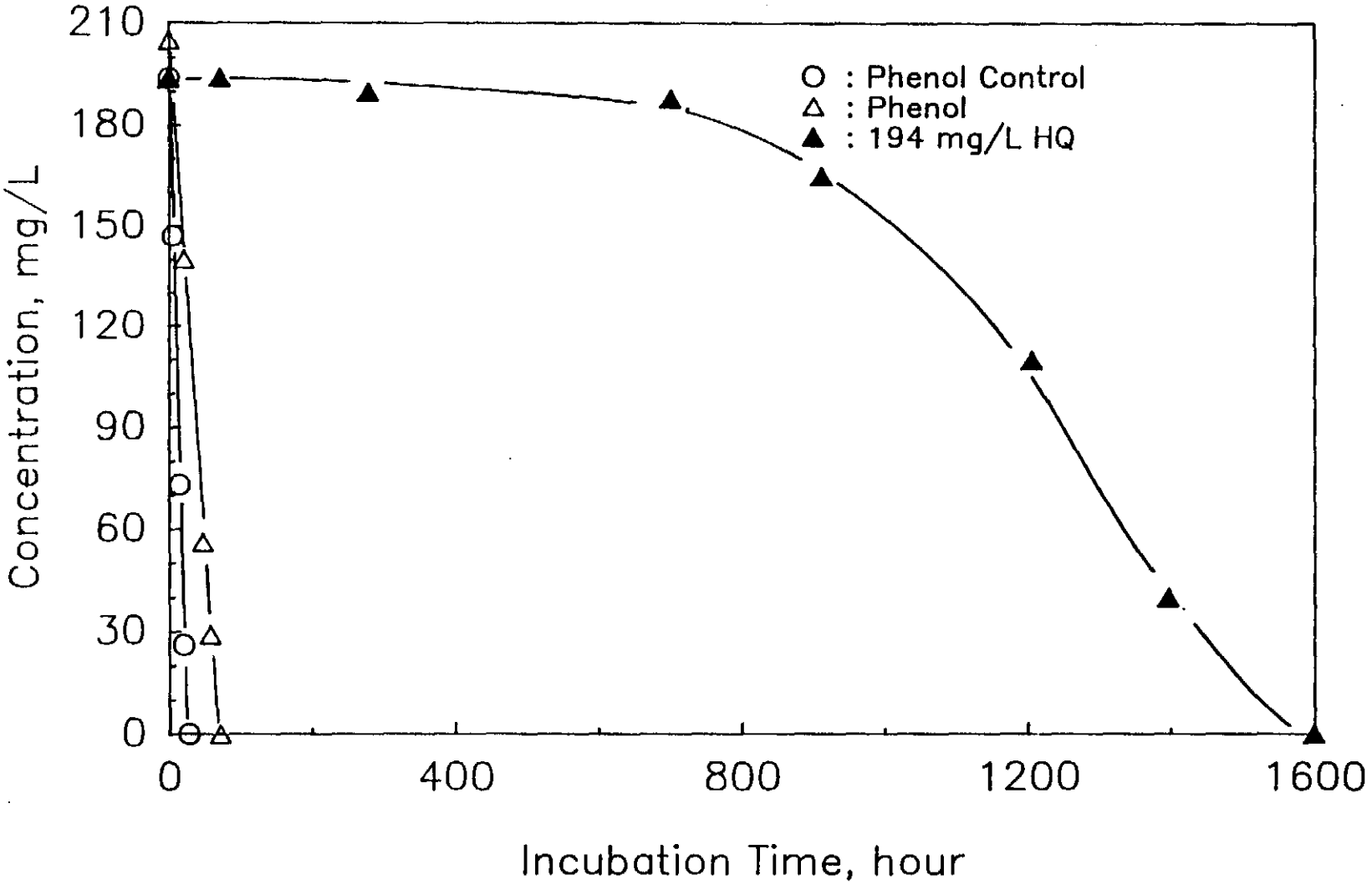

Figure 66. Fate of Hydroquinone in the Phenol-supplemented Culture: Initial Concentration of 194 mg/L

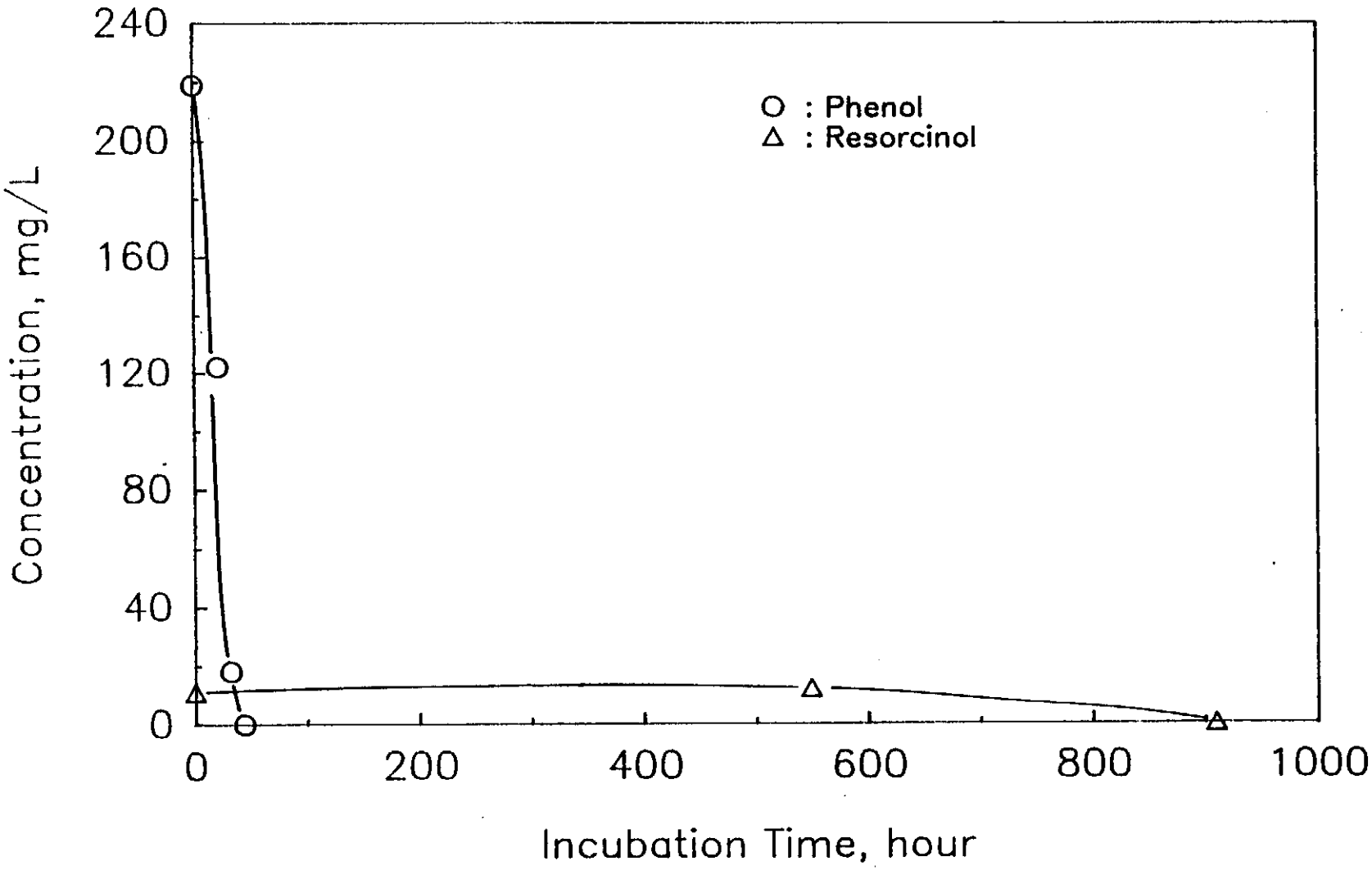

Figure 67. Fate of Resorcinol in the Phenol-supplemented Culture: Initial Concentration of 11 mg/L

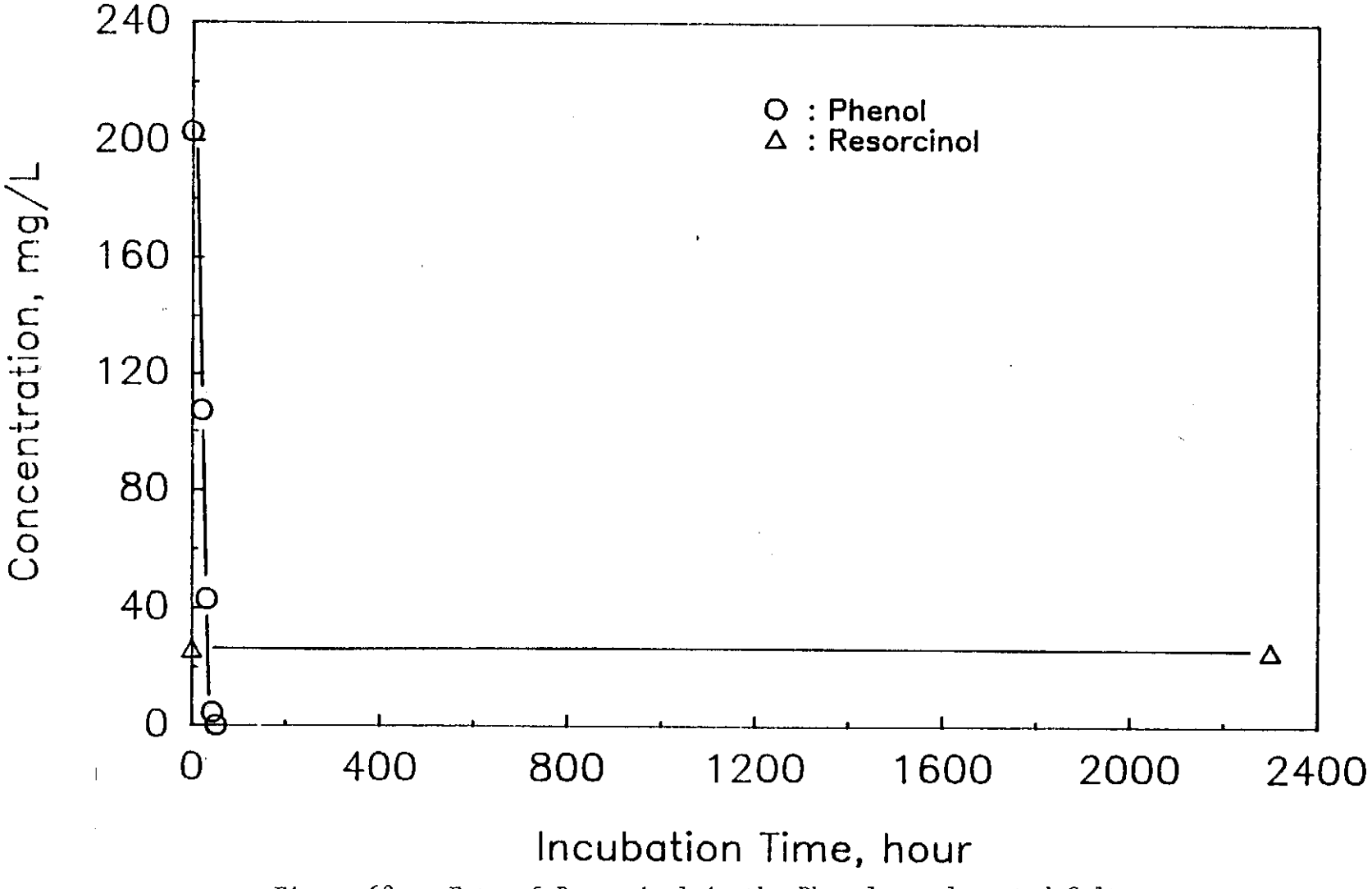

Figure 68. Fate of Resorcinol in the Phenol-supplemented Culture: Initial Concentration of 26 mg/L

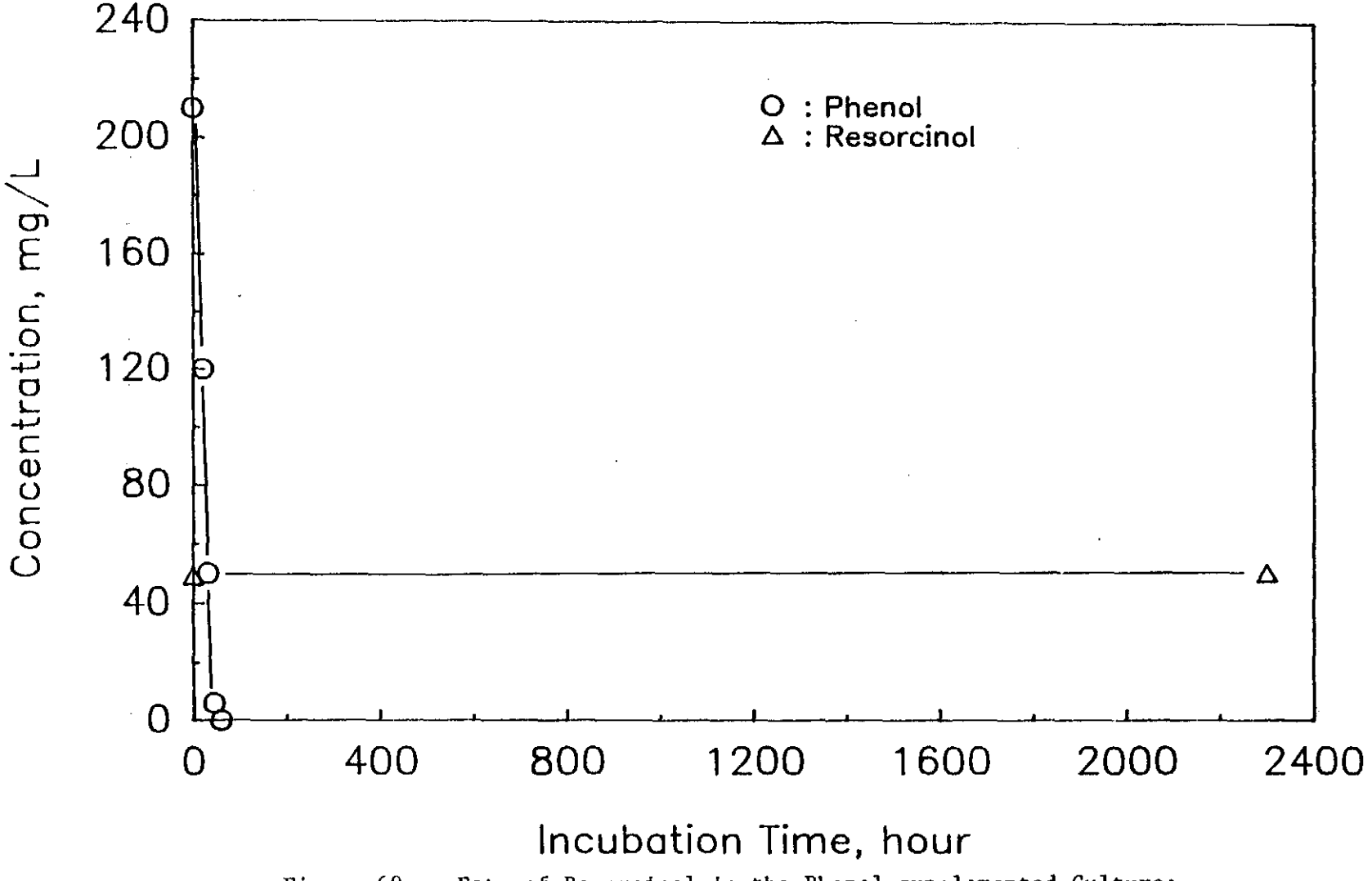

Figure 69. Fate of Resorcinol in the Phenol-supplemented Culture: Initial Concentration of 49 mg/L

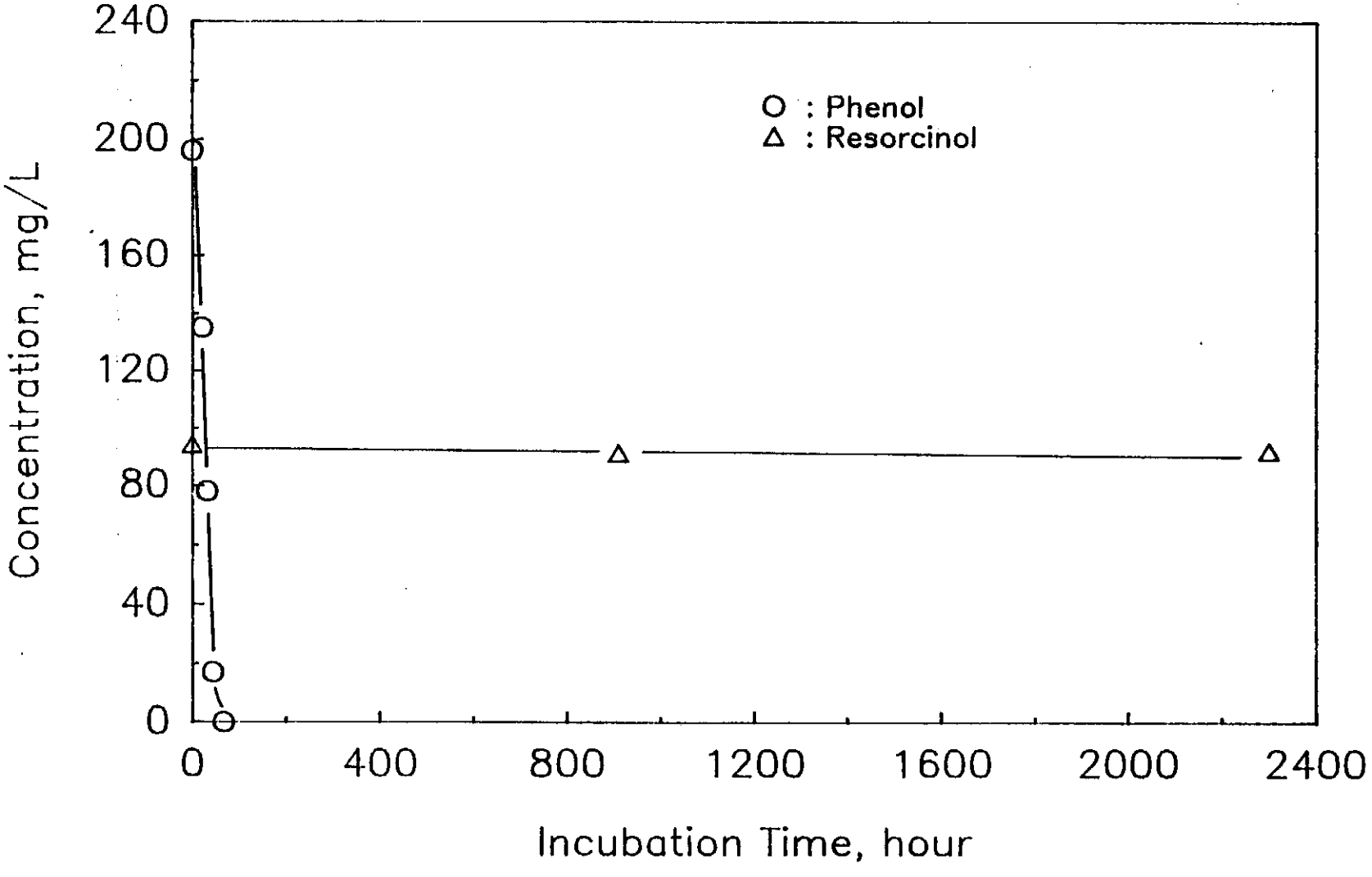

Figure 70. Fate of Resorcinol in the Phenol-supplemented Culture: Initial Concentration of 94 mg/L

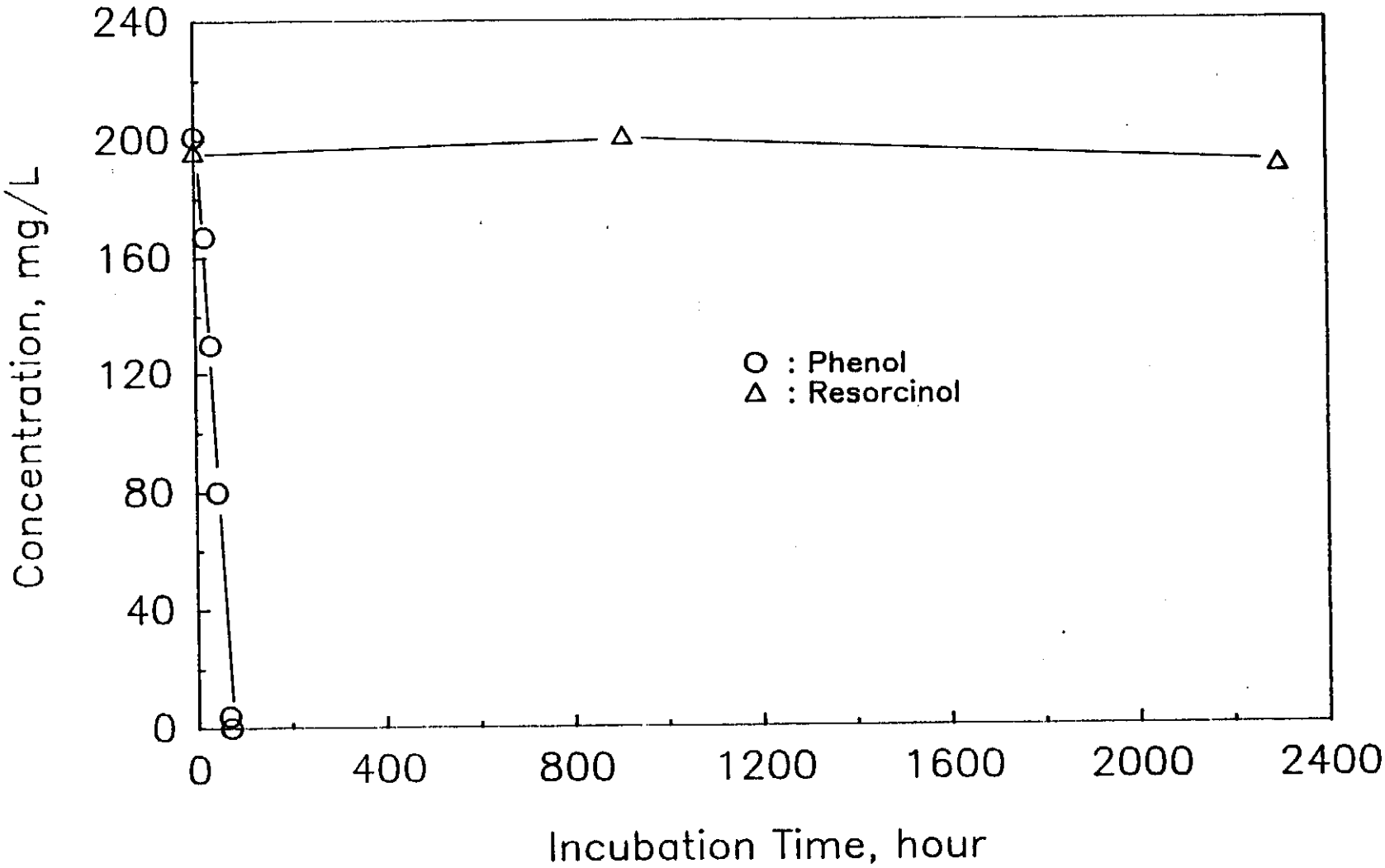

Figure 71. Fate of Resorcinol in the Phenol-supplemented Culture: Initial Concentration of 196 mg/L

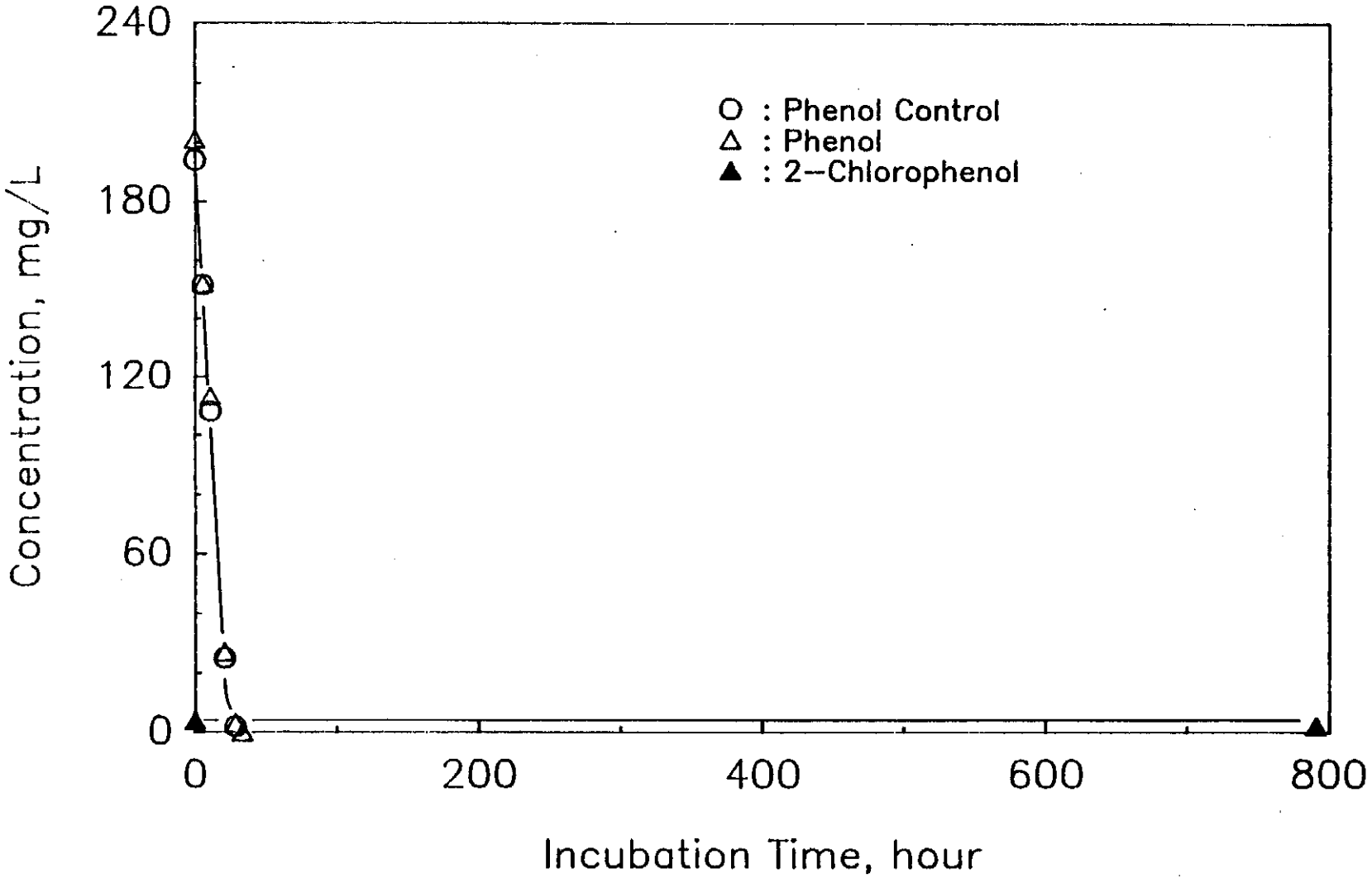

Figure 72. Fate of 2-CP in the Phenol-supplemented Culture: Initial Concentration of 4 mg/L

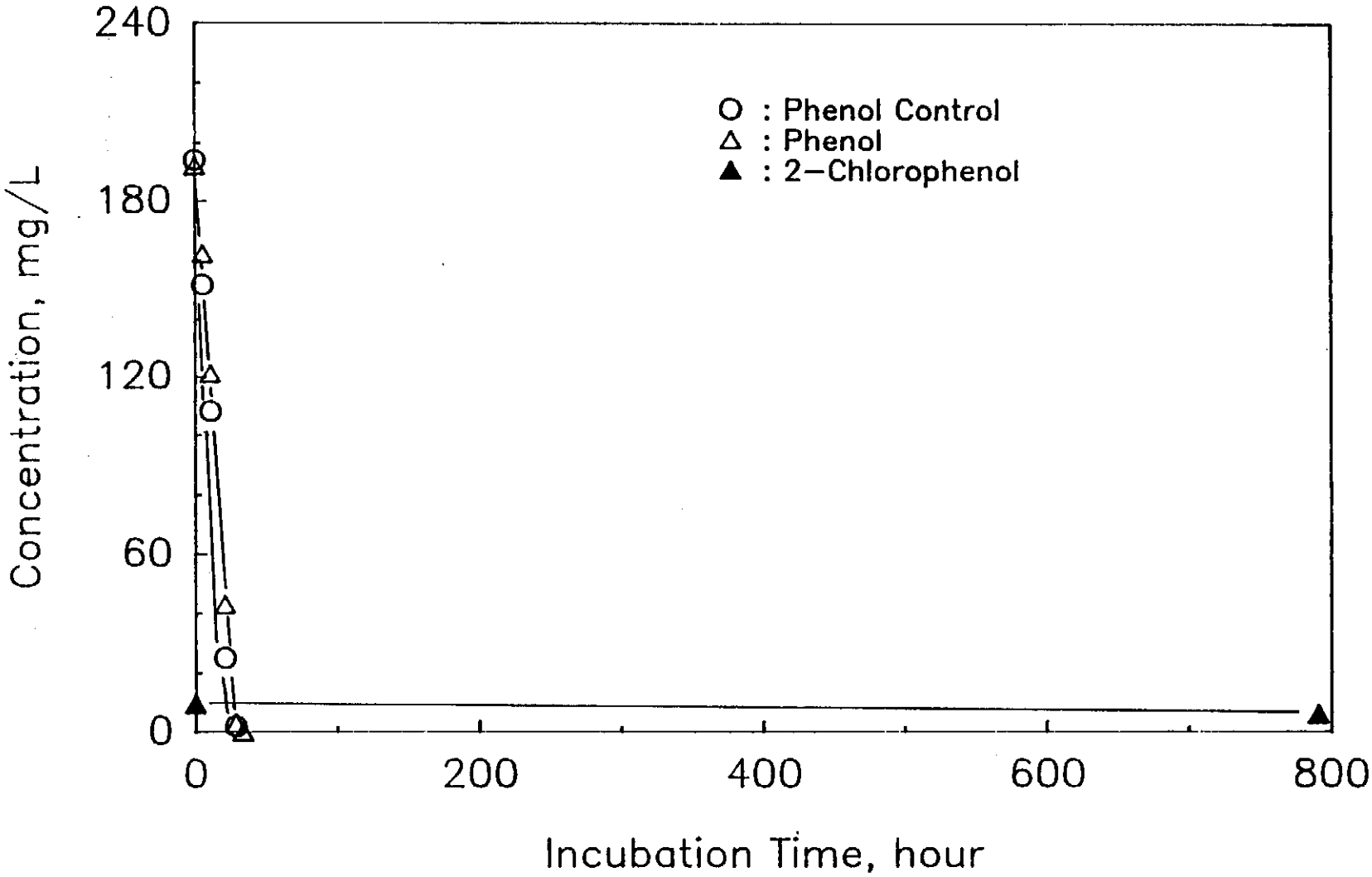

Figure 73. Fate of 2-CP in the Phenol-supplemented Culture: Initial Concentration of 10 mg/L

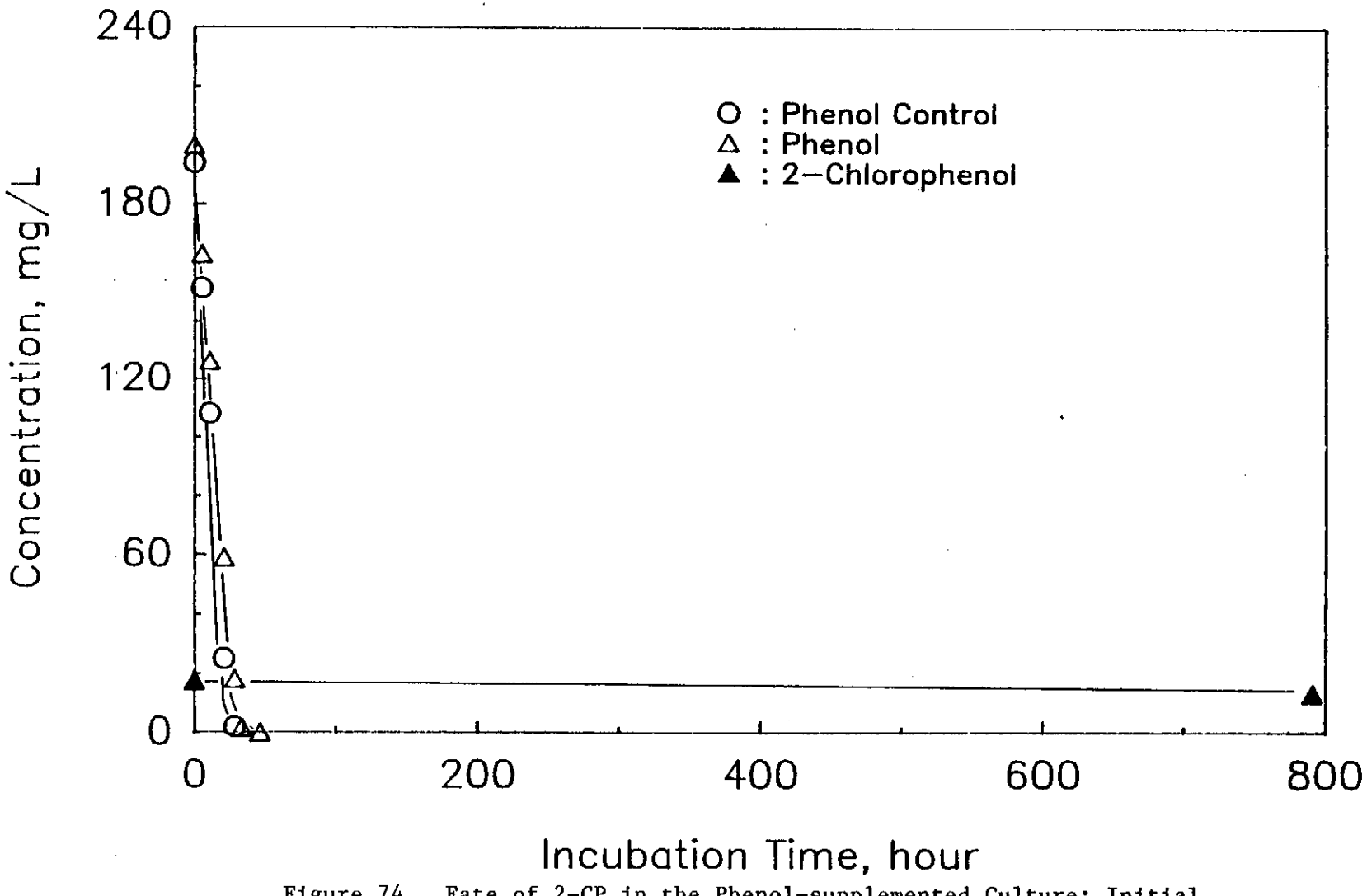

Figure 74. Fate of 2-CP in the Phenol-supplemented Culture: Initial Concentration of 18 mg/L

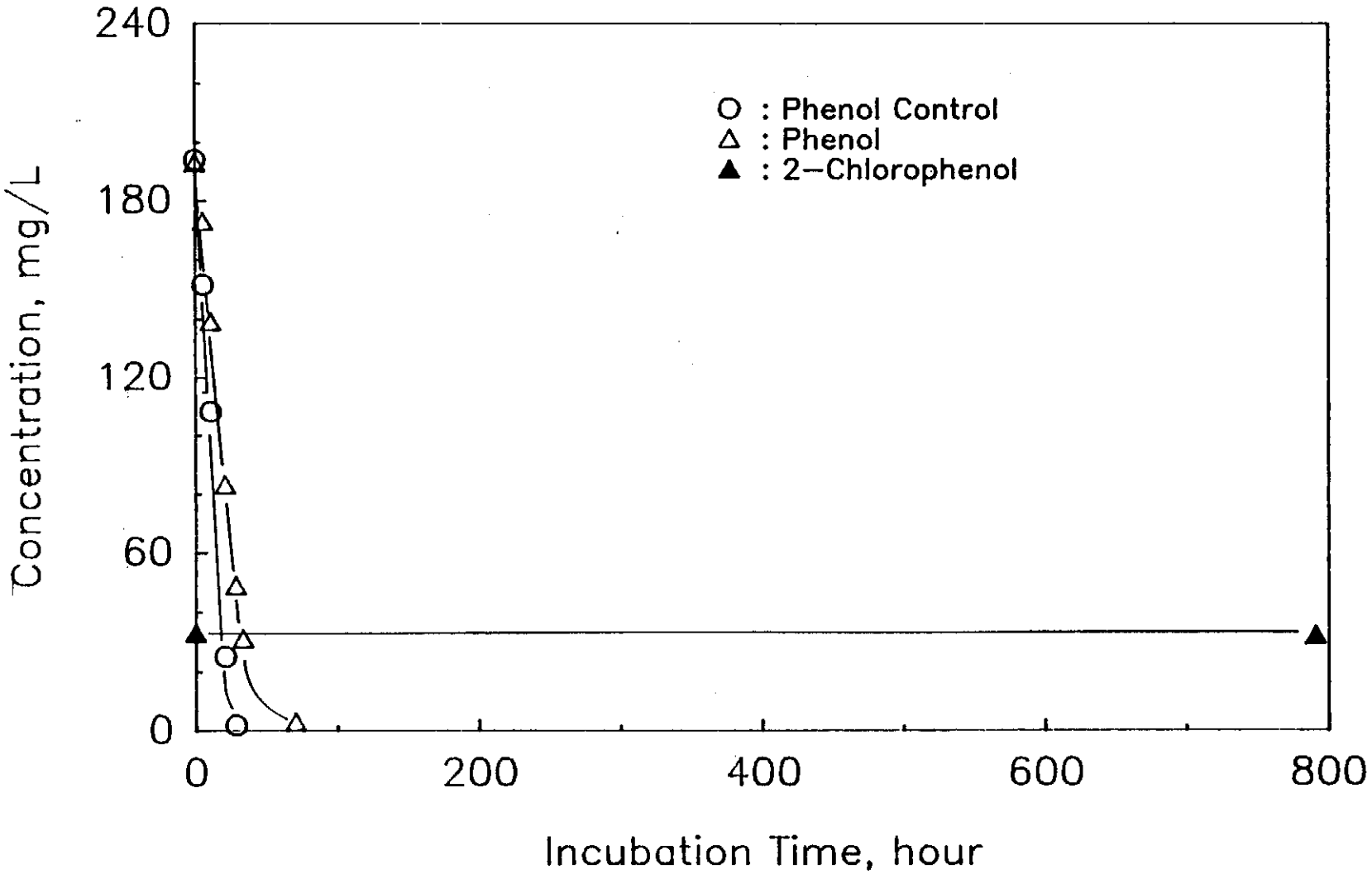

Figure 75. Fate of 2-CP in the Phenol-supplemented Culture: Initial Concentration of 33 mg/L

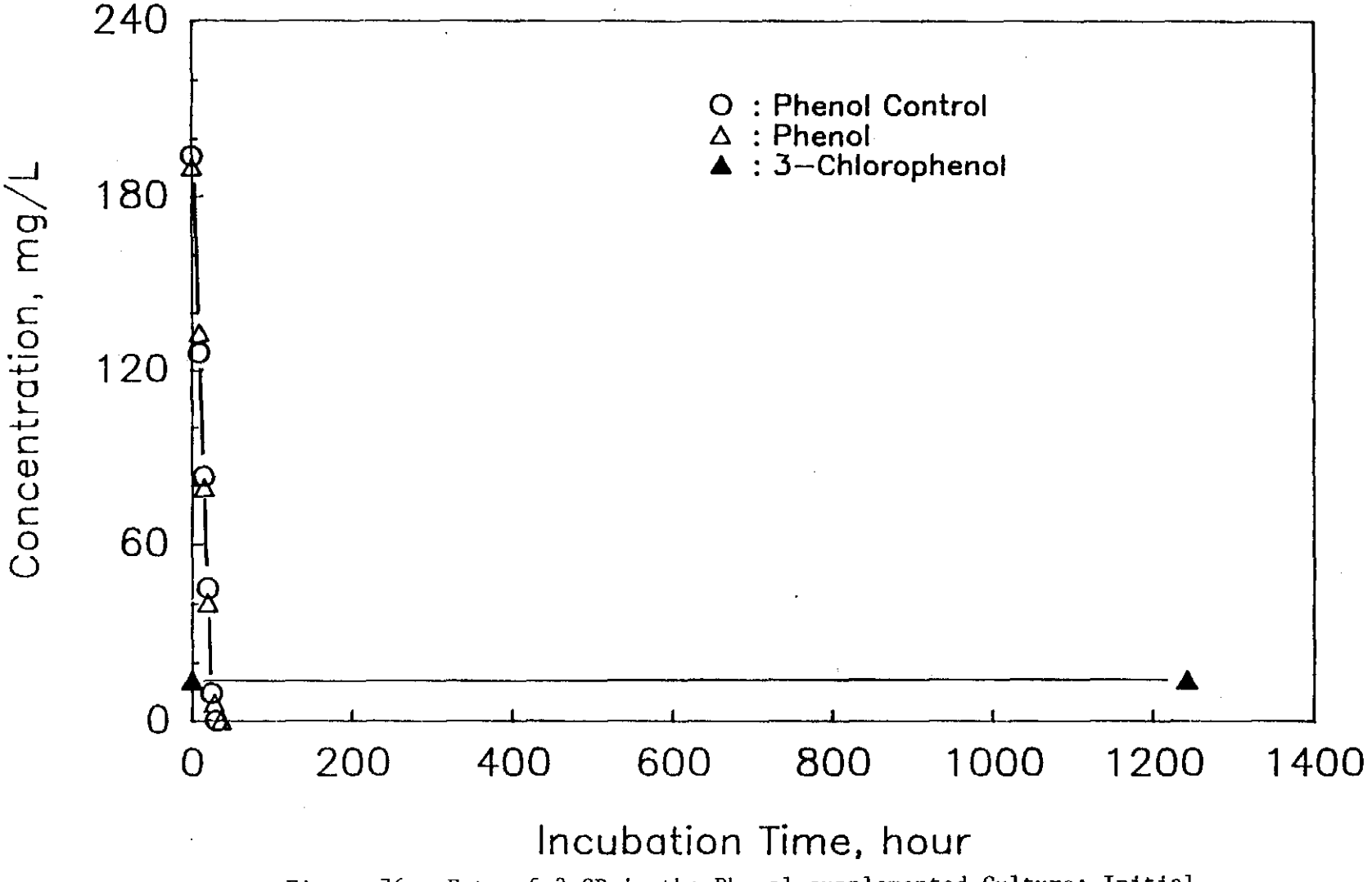

Figure 76. Fate of 3-CP in the Phenol-supplemented Culture: Initial Concentration of 13 mg/L

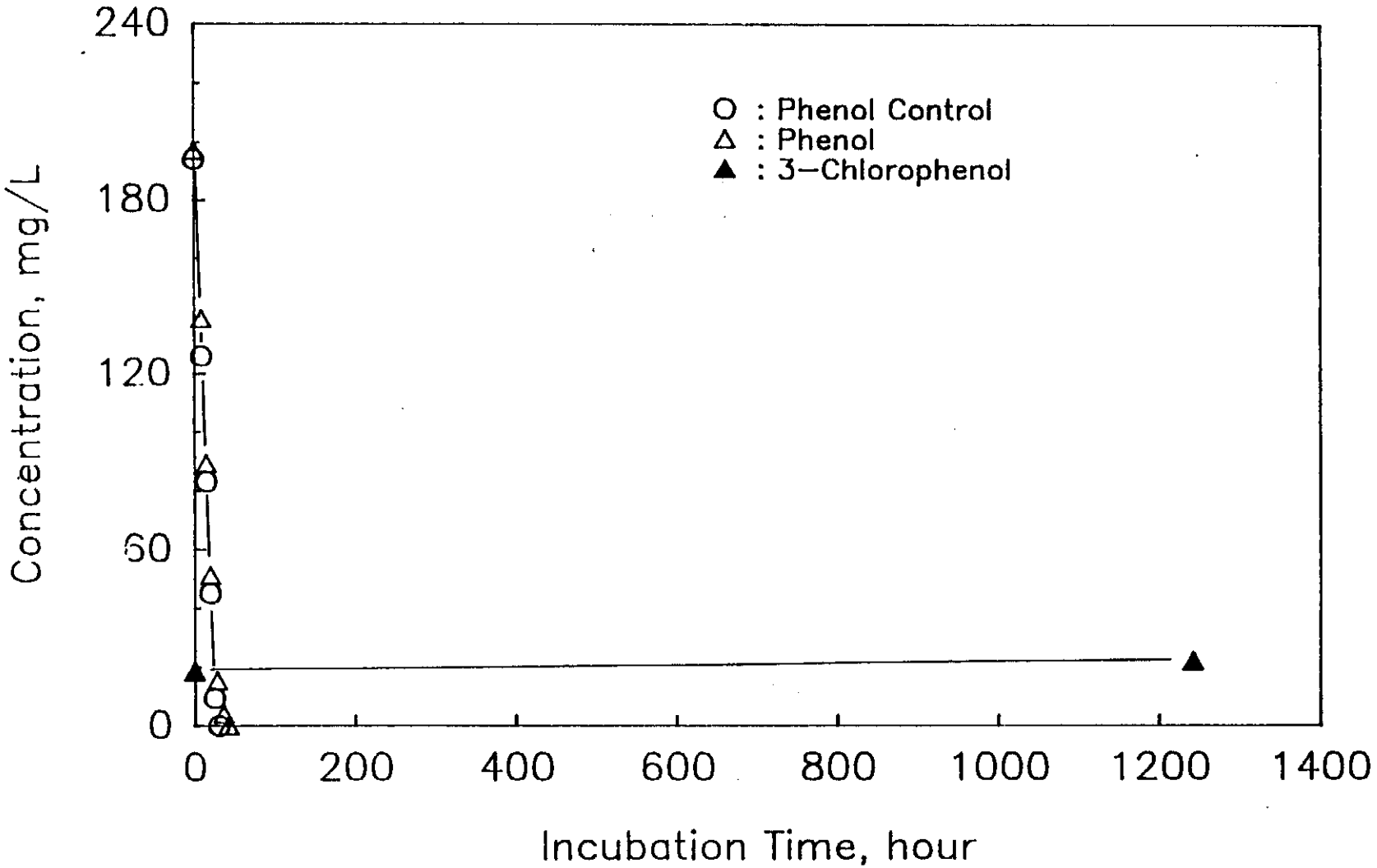

Figure 77. Fate of 3-CP in the Phenol-supplemented Culture: Initial Concentration of 18 mg/L

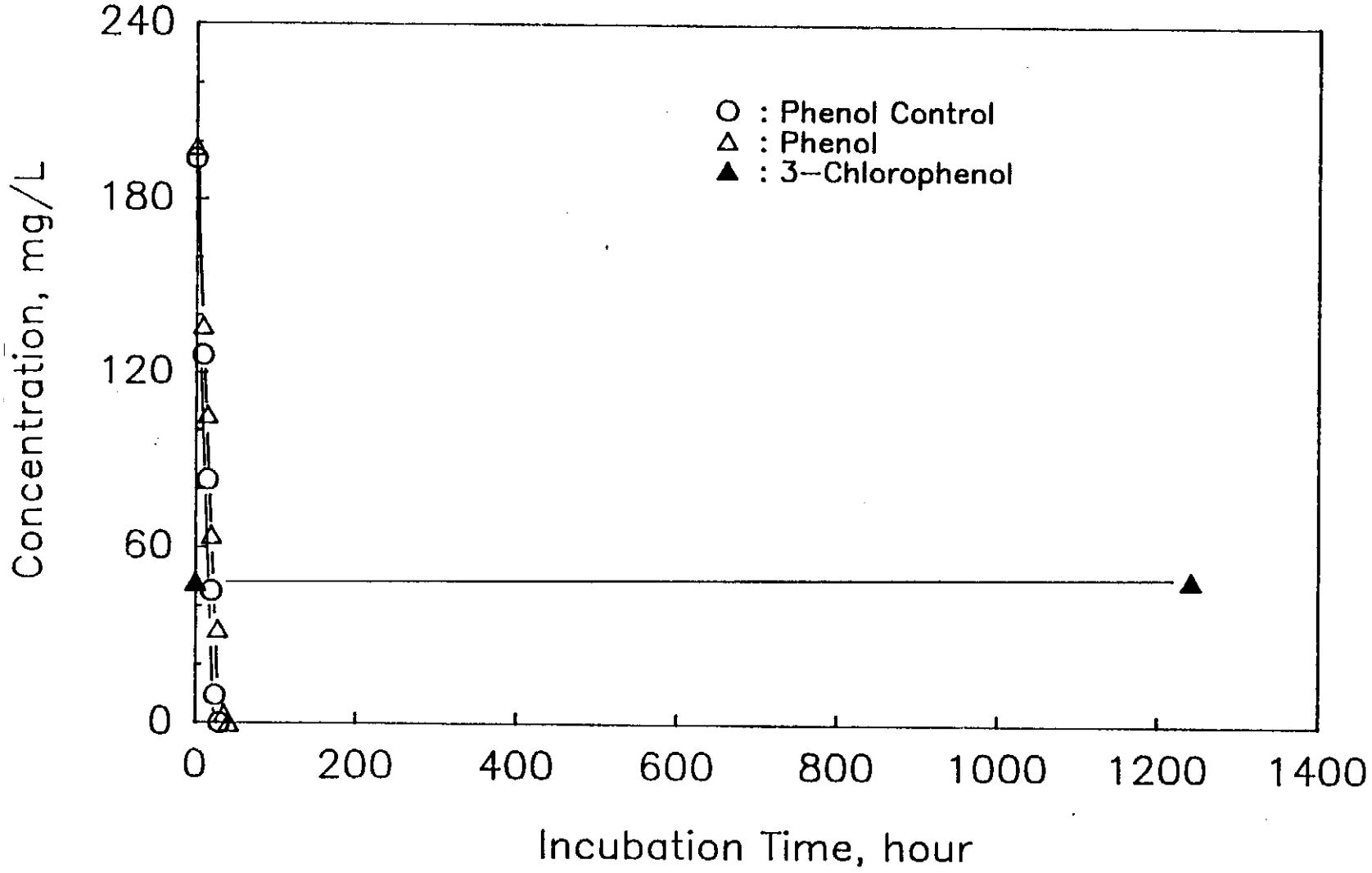

Figure 78. Fate of 3-CP in the Phenol-supplemented Culture: Initial Concentration of 48 mg/L

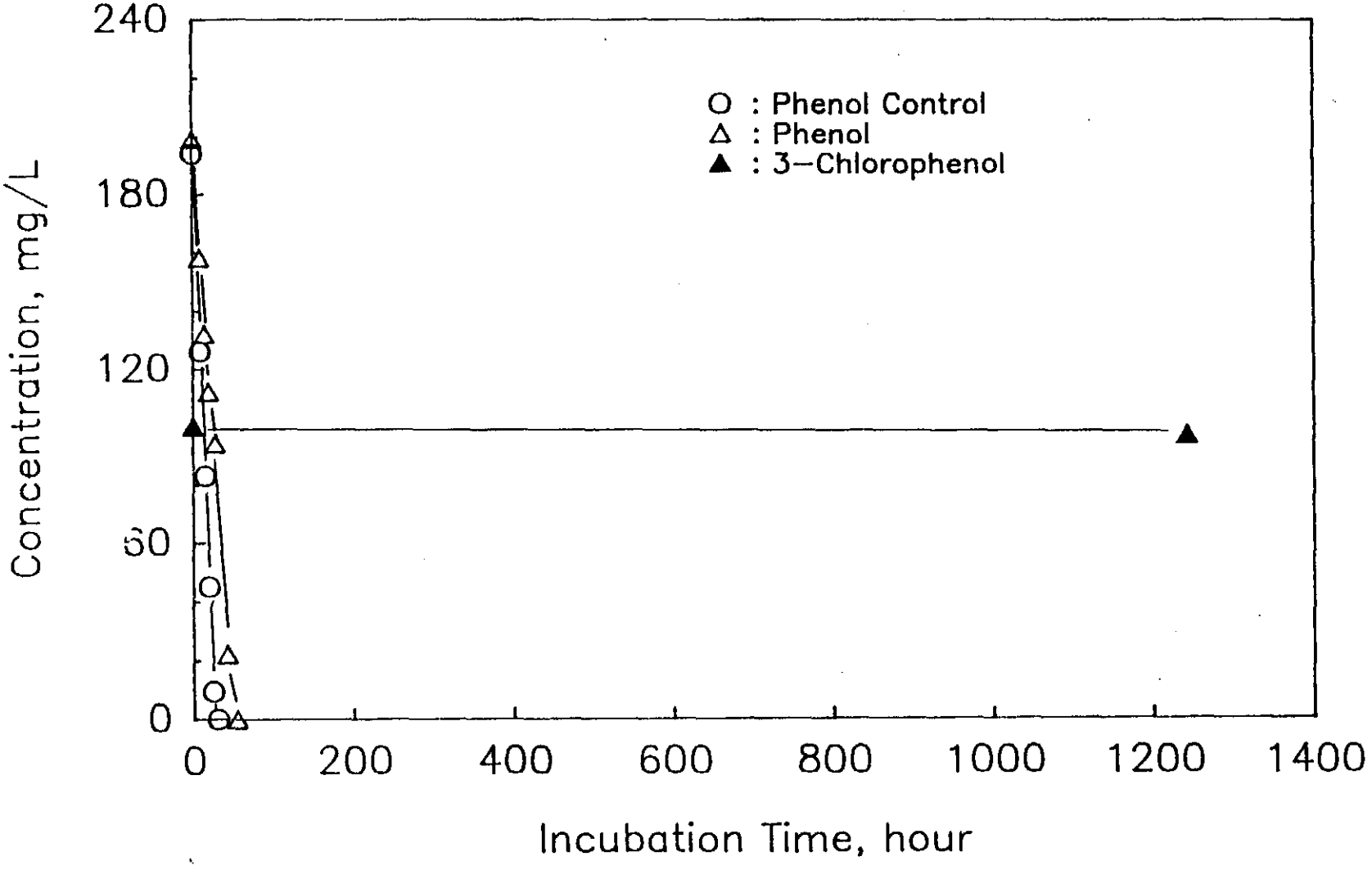

Figure 79. Fate of 3-CP in the Phenol-supplemented Culture: Initial Concentration of 100 mg/L

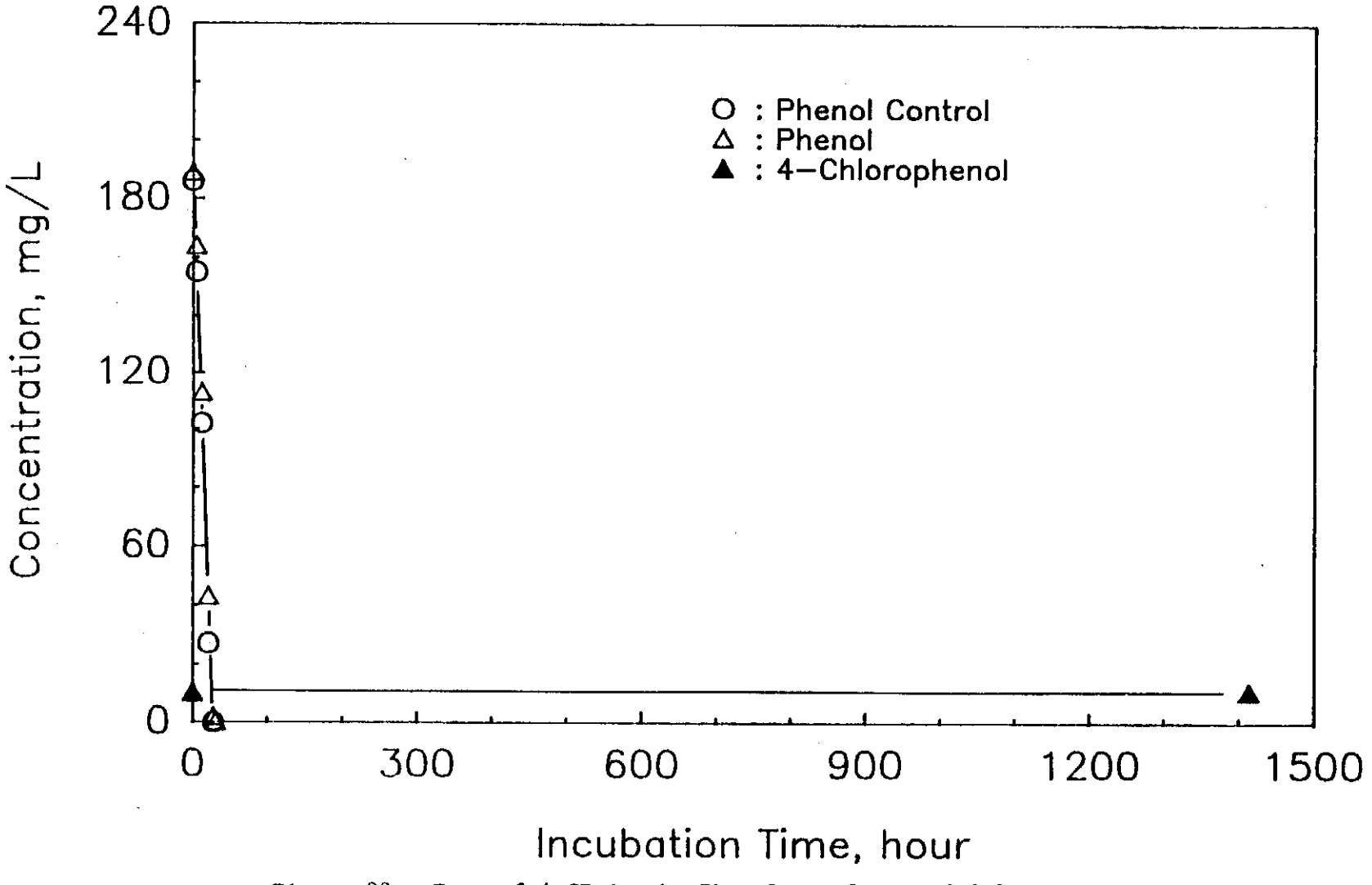

Figure 80. Fate of 4-CP in the Phenol-supplemented Culture: Initial Concentration of 10 mg/L

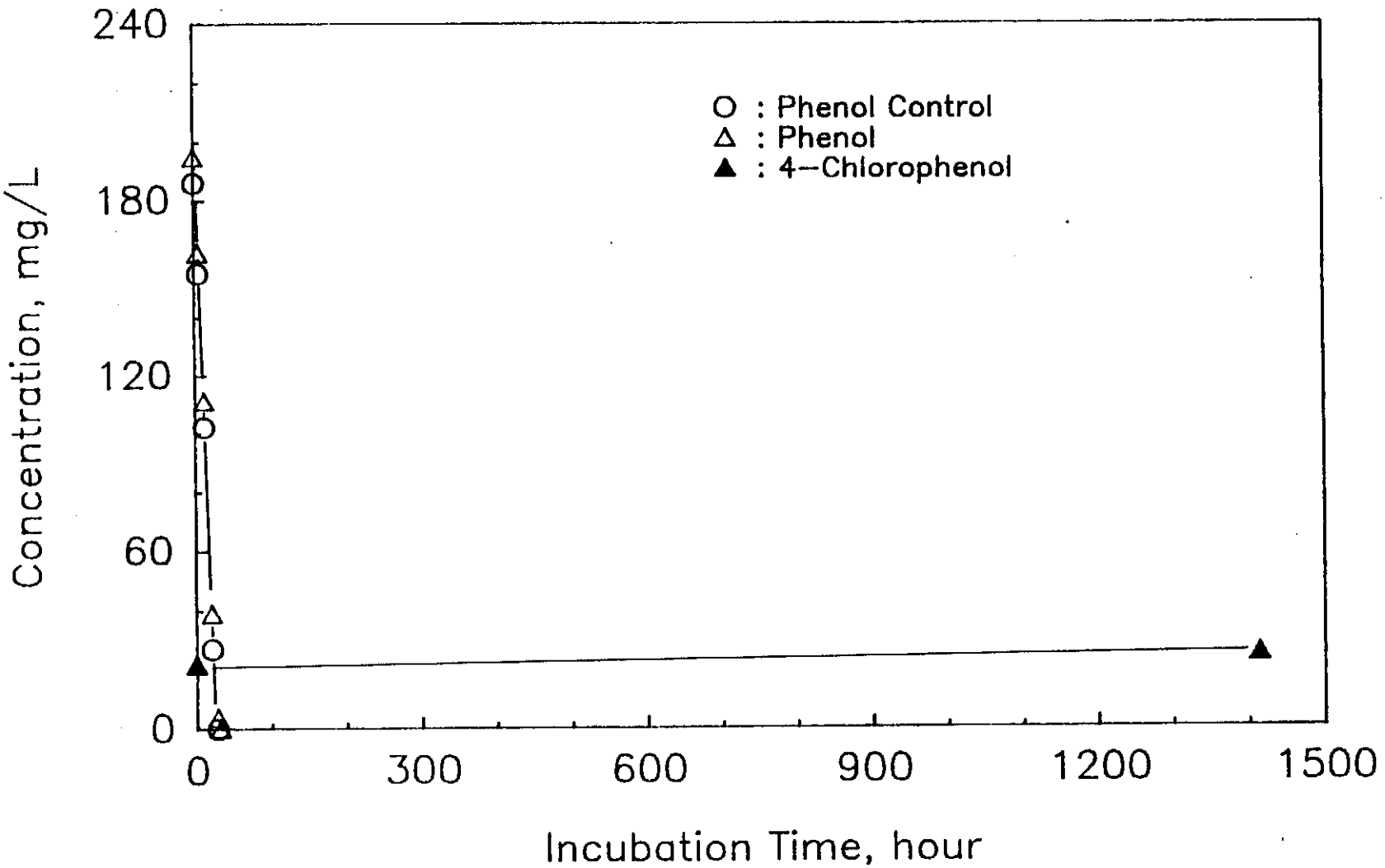

Figure 81. Fate of 4-CP in the Phenol-supplemented Culture: Initial Concentration of 22 mg/L

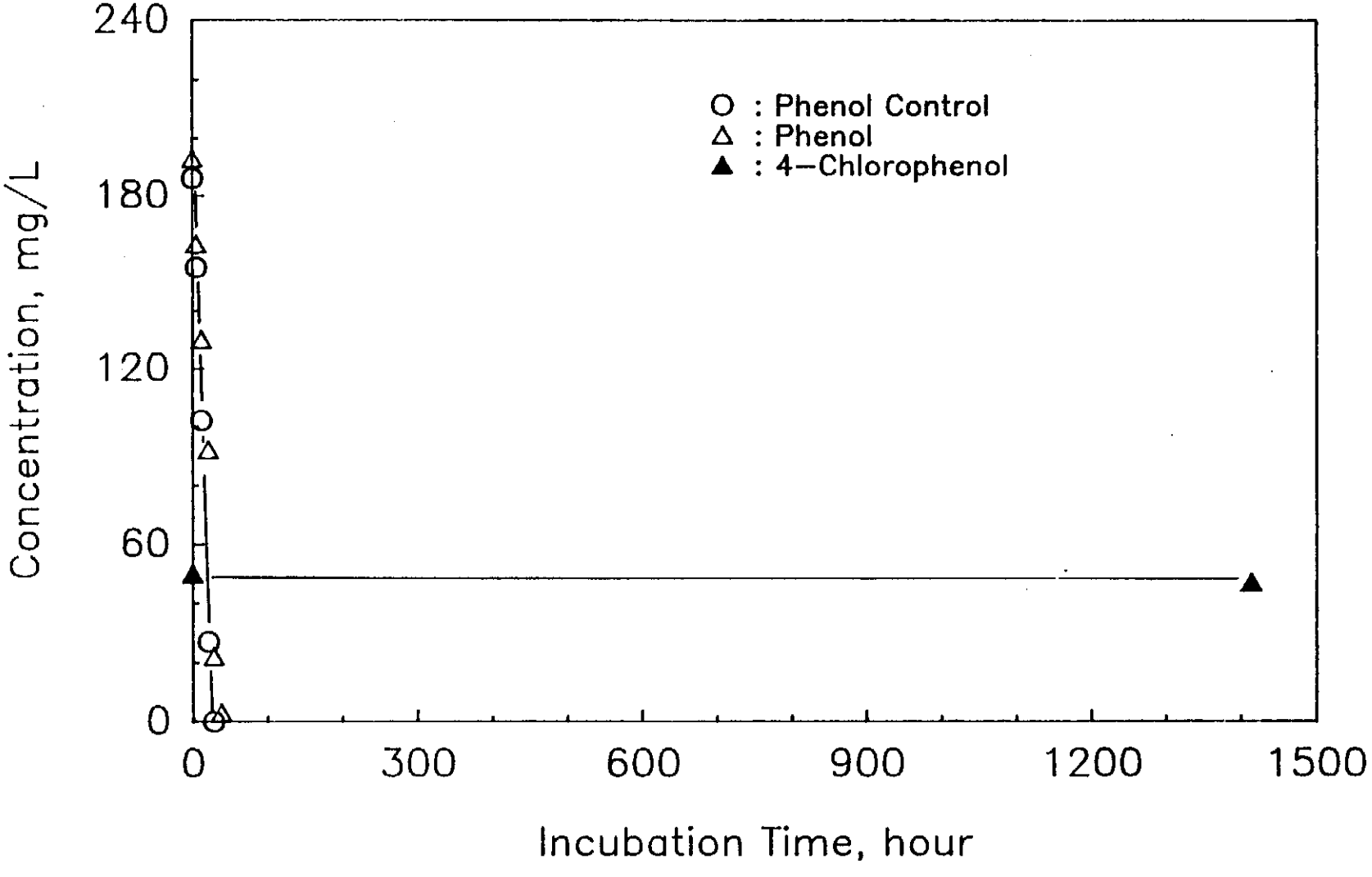

Figure 82. Fate of 4-CP in the Phenol-supplemented Culture: Initial Concentration of 42 mg/L

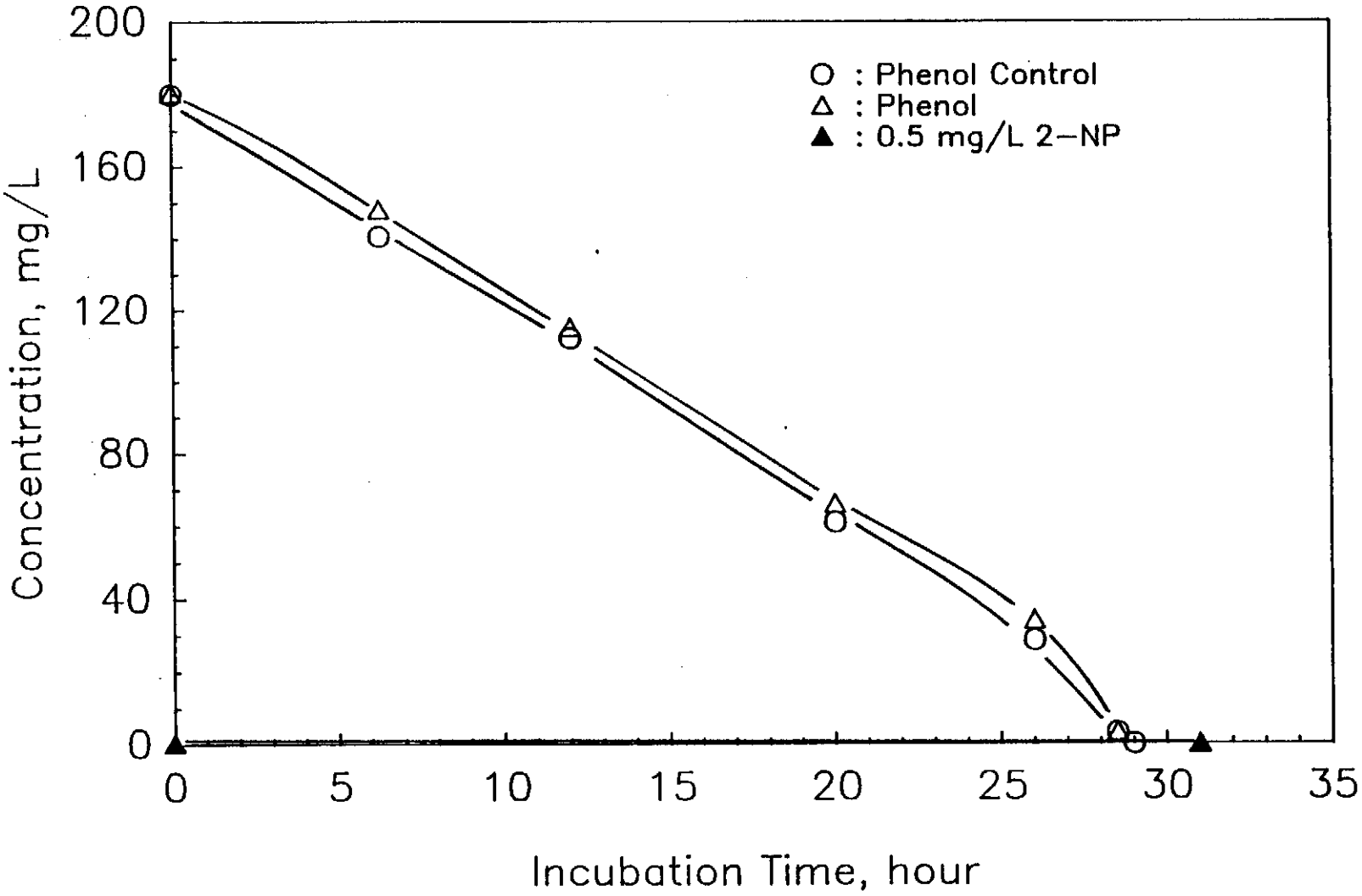

Figure 83. Fate of 2-NP in the Phenol-supplemented Culture: Initial Concentration of 0.5 mg/L

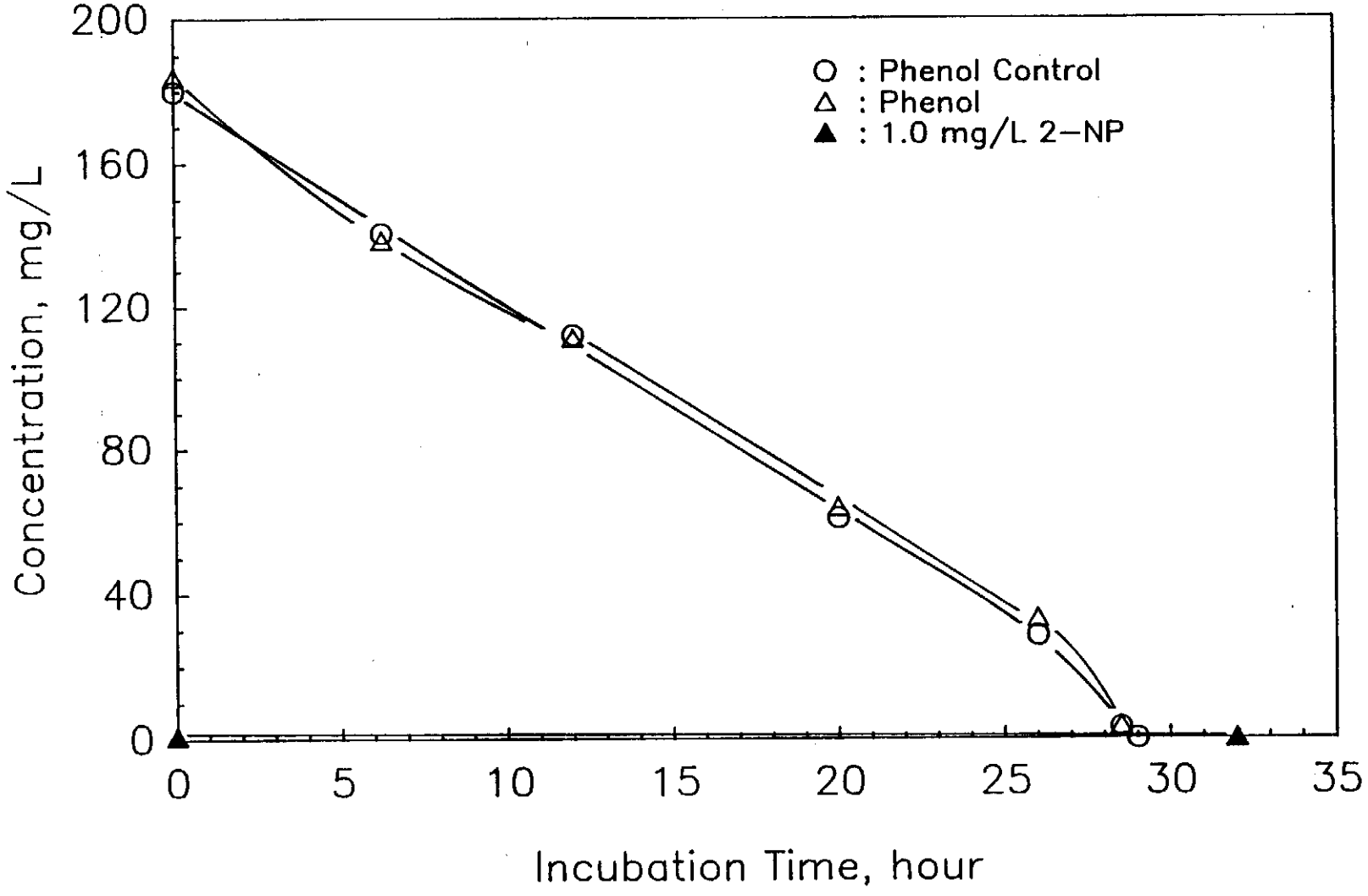

Figure 84. Fate of 2-NP in the Phenol-supplemented Culture: Initial Concentration of 1 mg/L

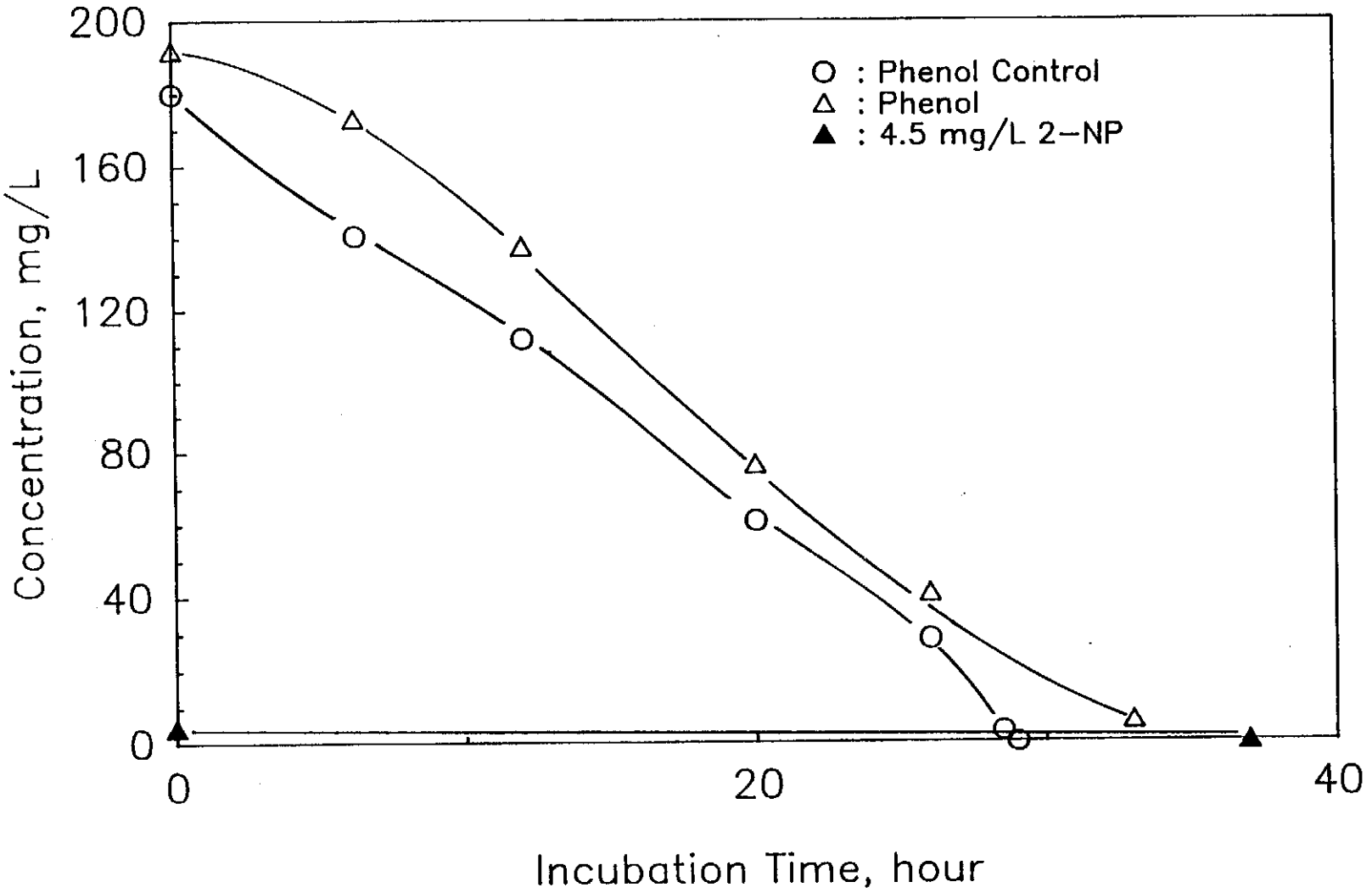

Figure 85. Fate of 2-NP in the Phenol-supplemented Culture: Initial Concentration of 5 mg/L

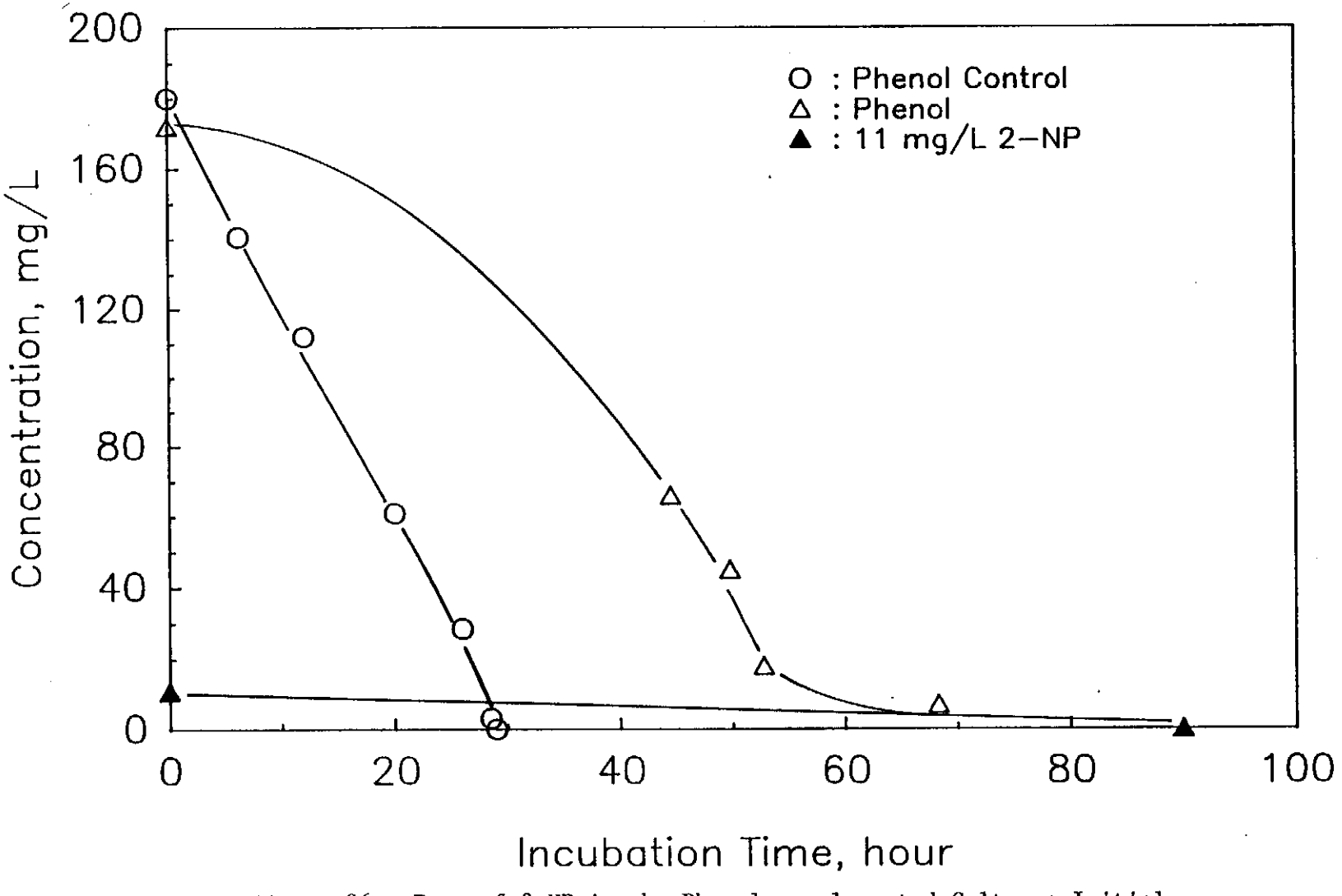

Figure 86. Fate of 2-NP in the Phenol-supplemented Culture: Initial Concentration of 11 mg/L

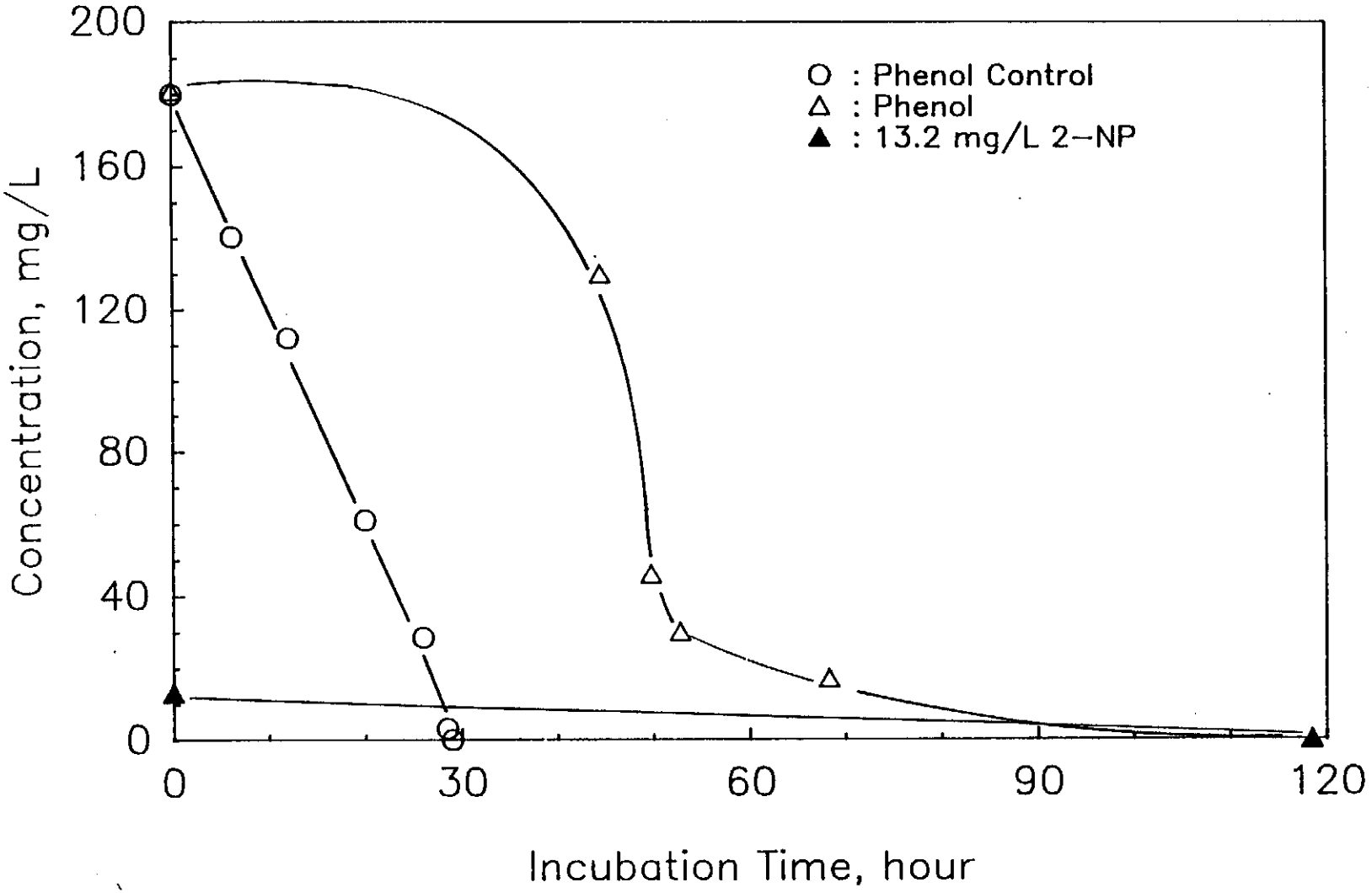

Figure 87. Fate of 2-NP in the Phenol-supplemented Culture: Initial Concentration of 13 mg/L

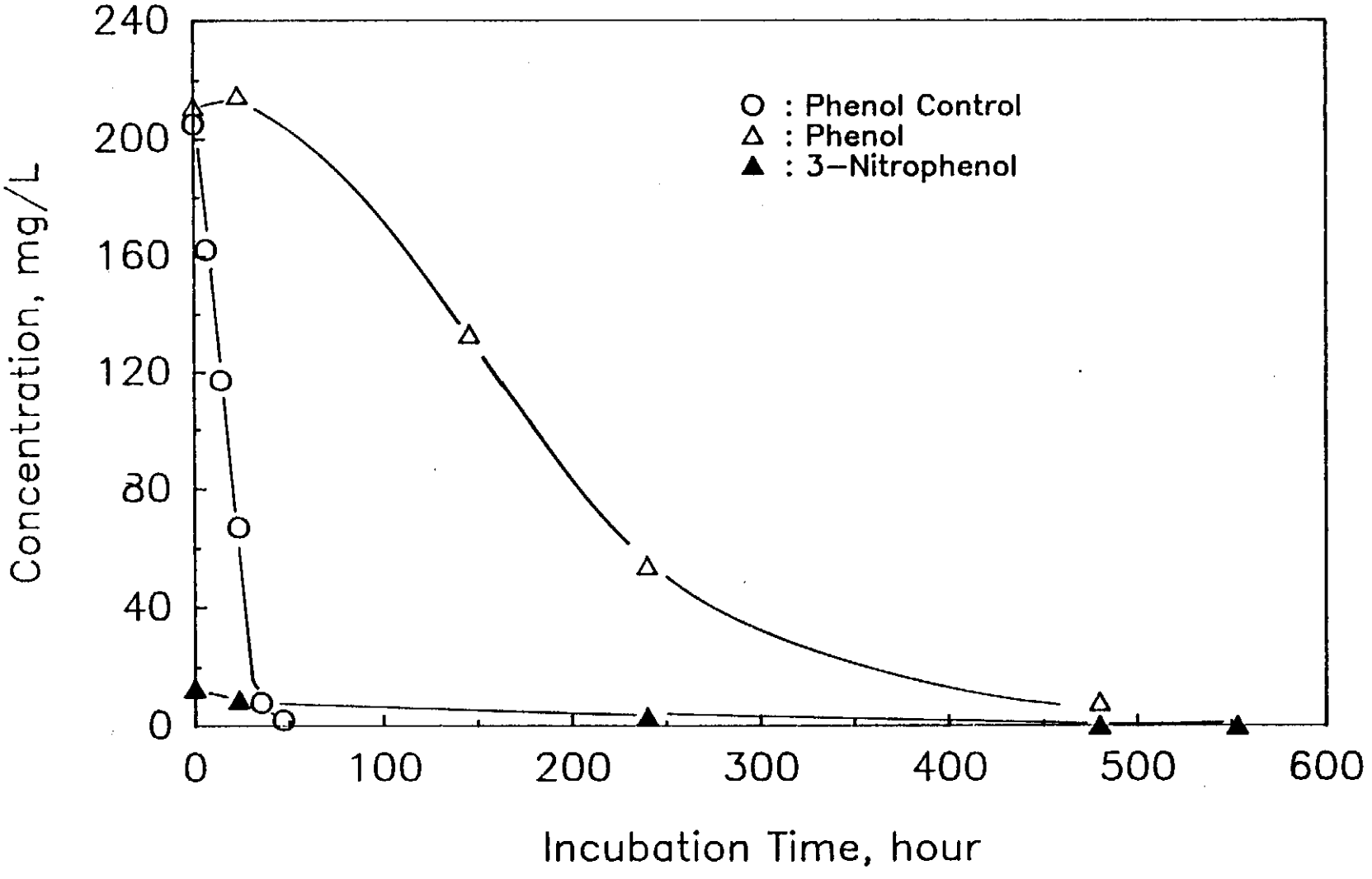

Figure 88. Fate of 3-NP in the Phenol-supplemented Culture: Initial Concentration of 12 mg/L

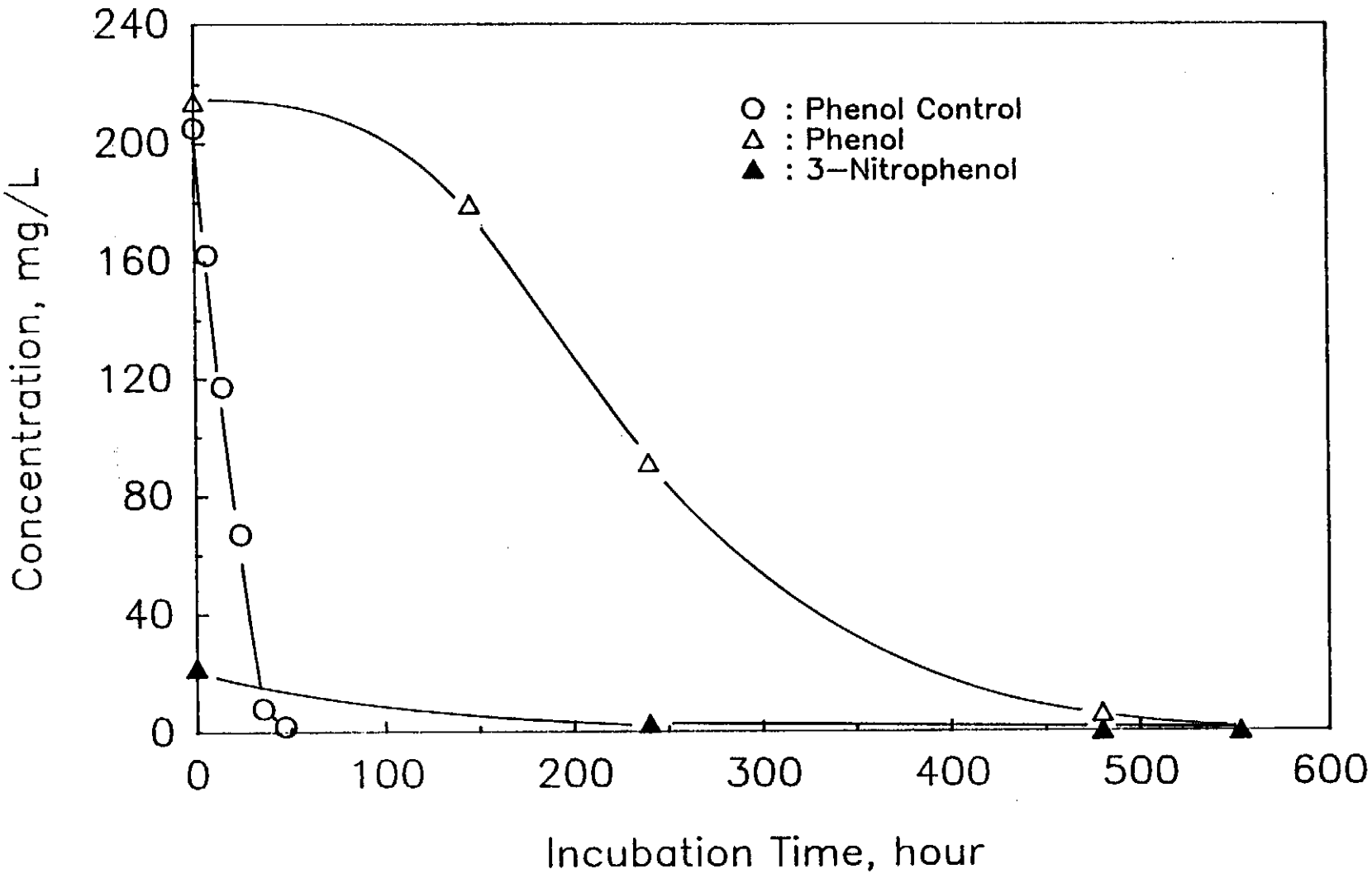

Figure 89. Fate of 3-NP in the Phenol-supplemented Culture: Initial Concentration of 21 mg/L

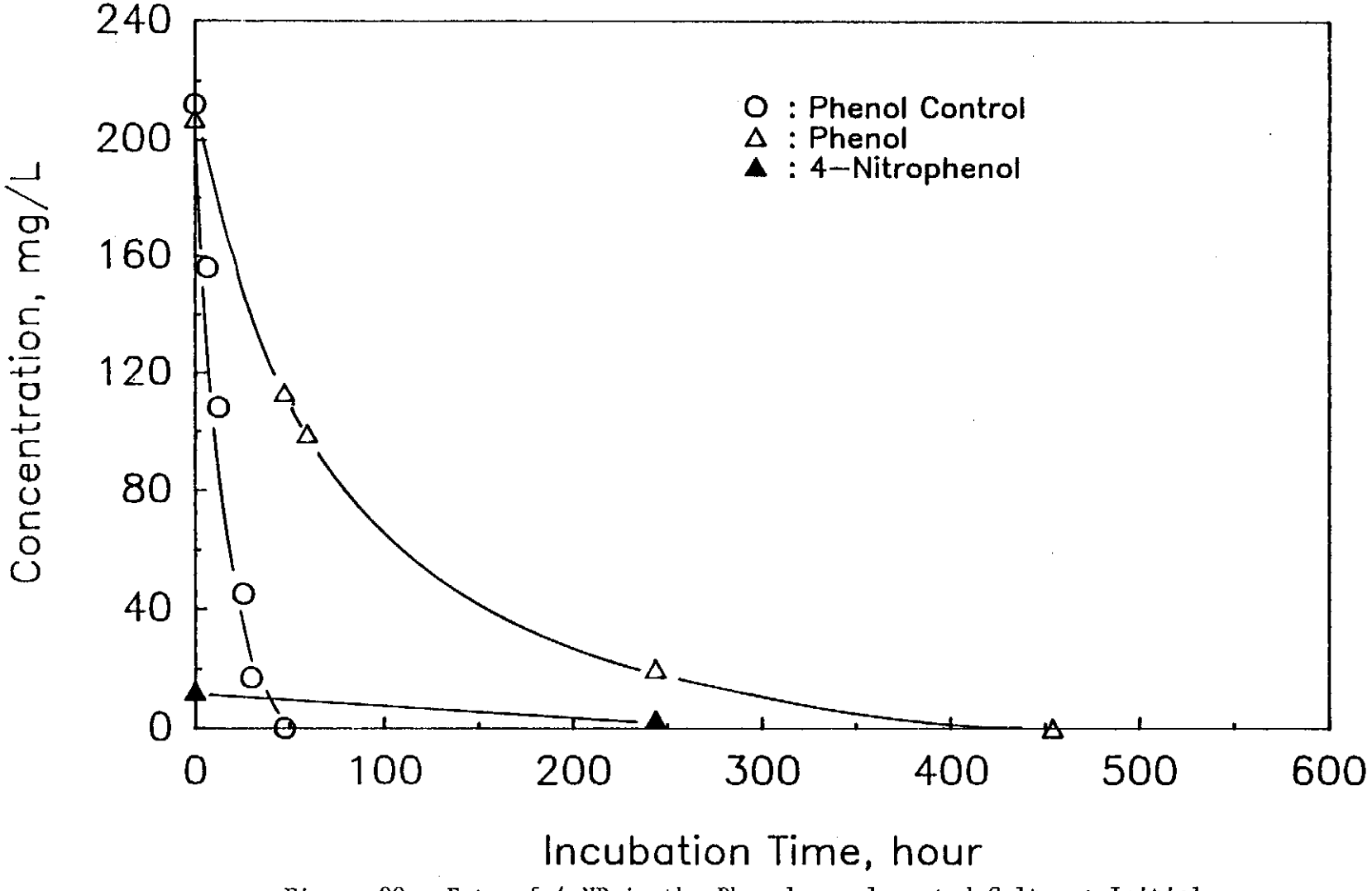

Figure 90. Fate of 4-NP in the Phenol-supplemented Culture: Initial Concentration of 8 mg/L

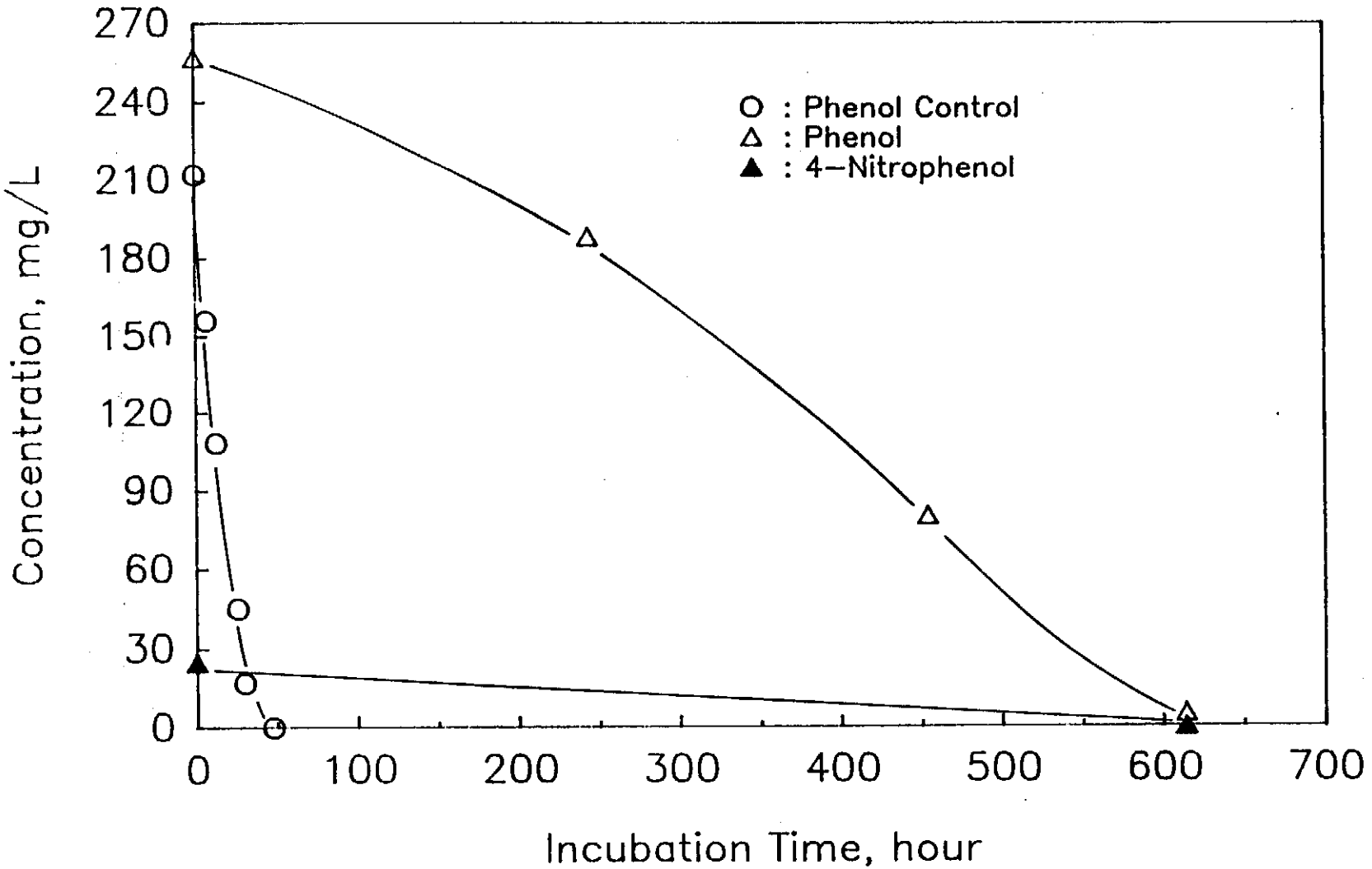

Figure 91. Fate of 4-NP in the Phenol-supplemented Culture: Initial Concentration of 22 mg/L

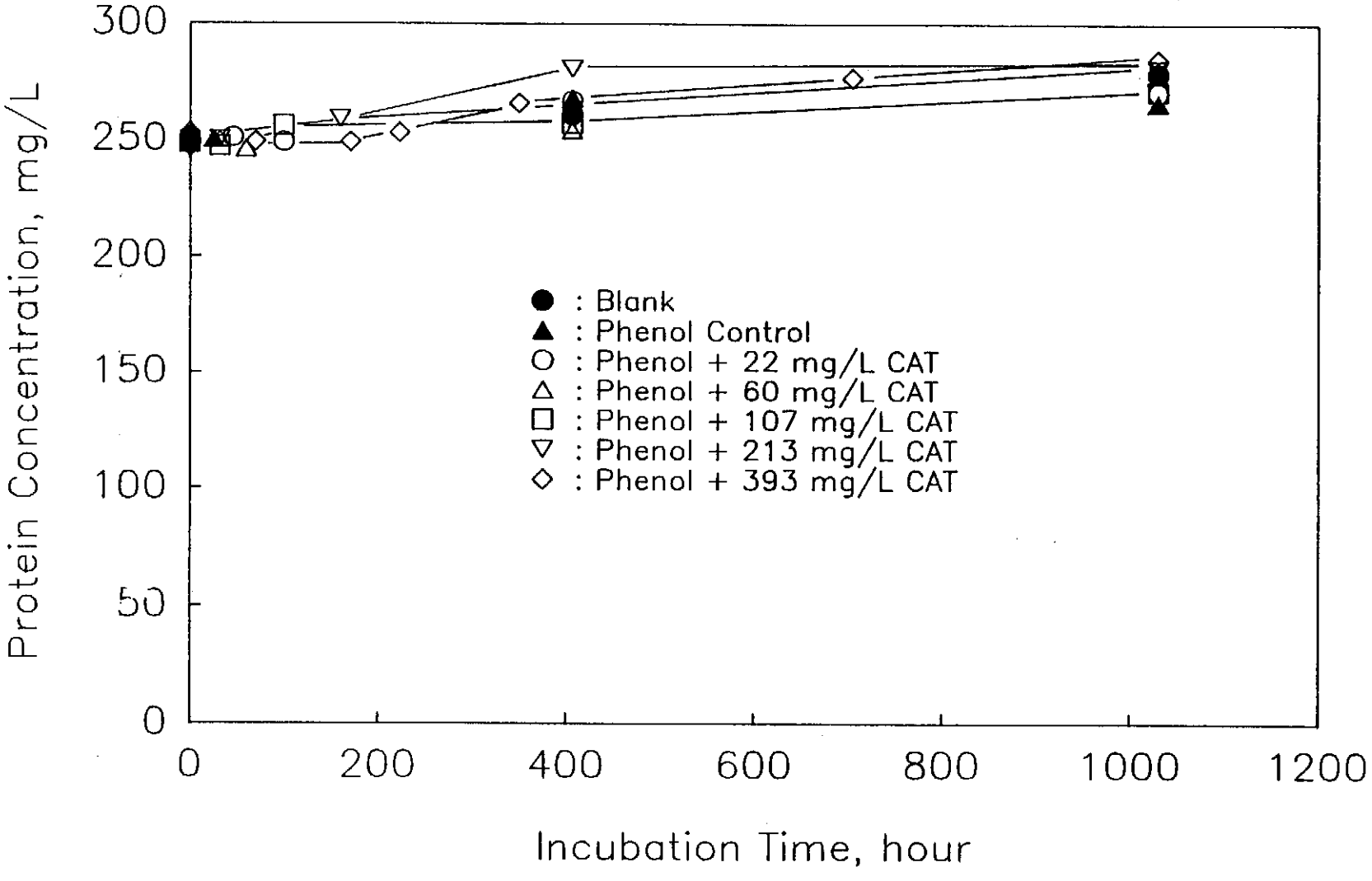

Figure 92. Growth of Biomass in the Phenol-supplemented Culture in the Presence of Catechol

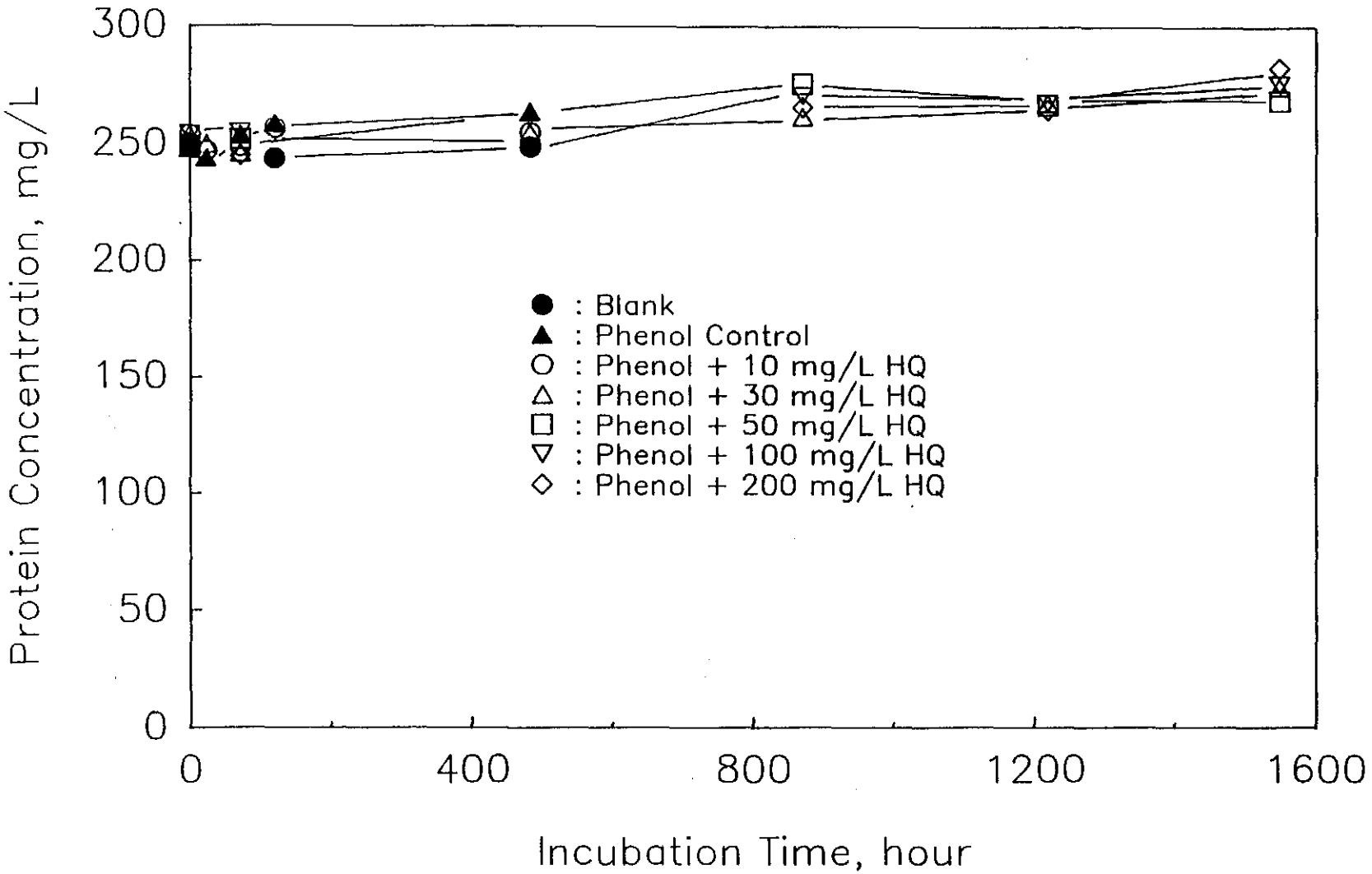

Figure 93. Growth of Biomass in the Phenol-supplemented Culture in the Presence of Hydroquinone

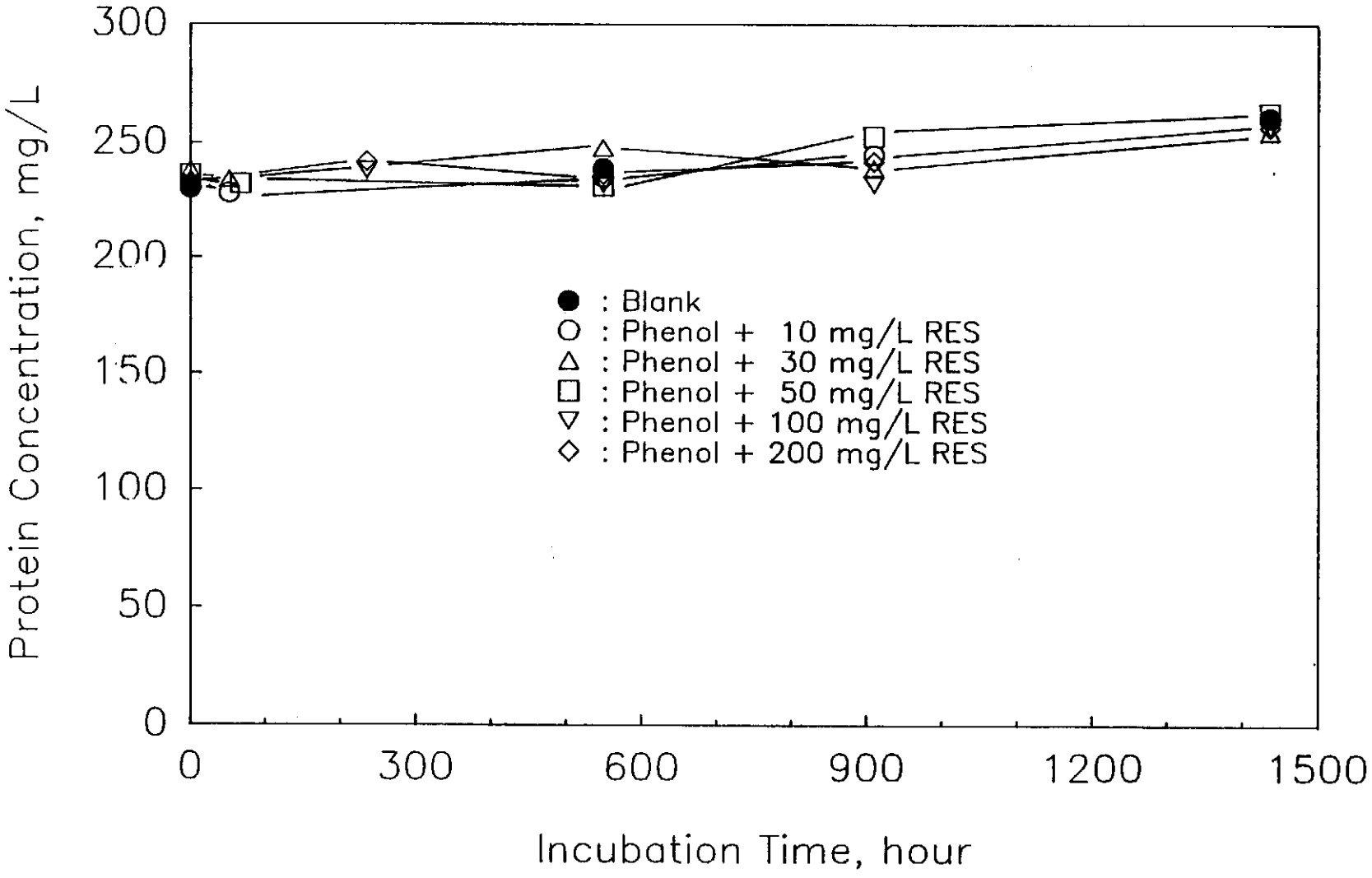

Figure 94. Growth of Biomass in the Phenol-supplemented Culture in the Presence of Resorcinol

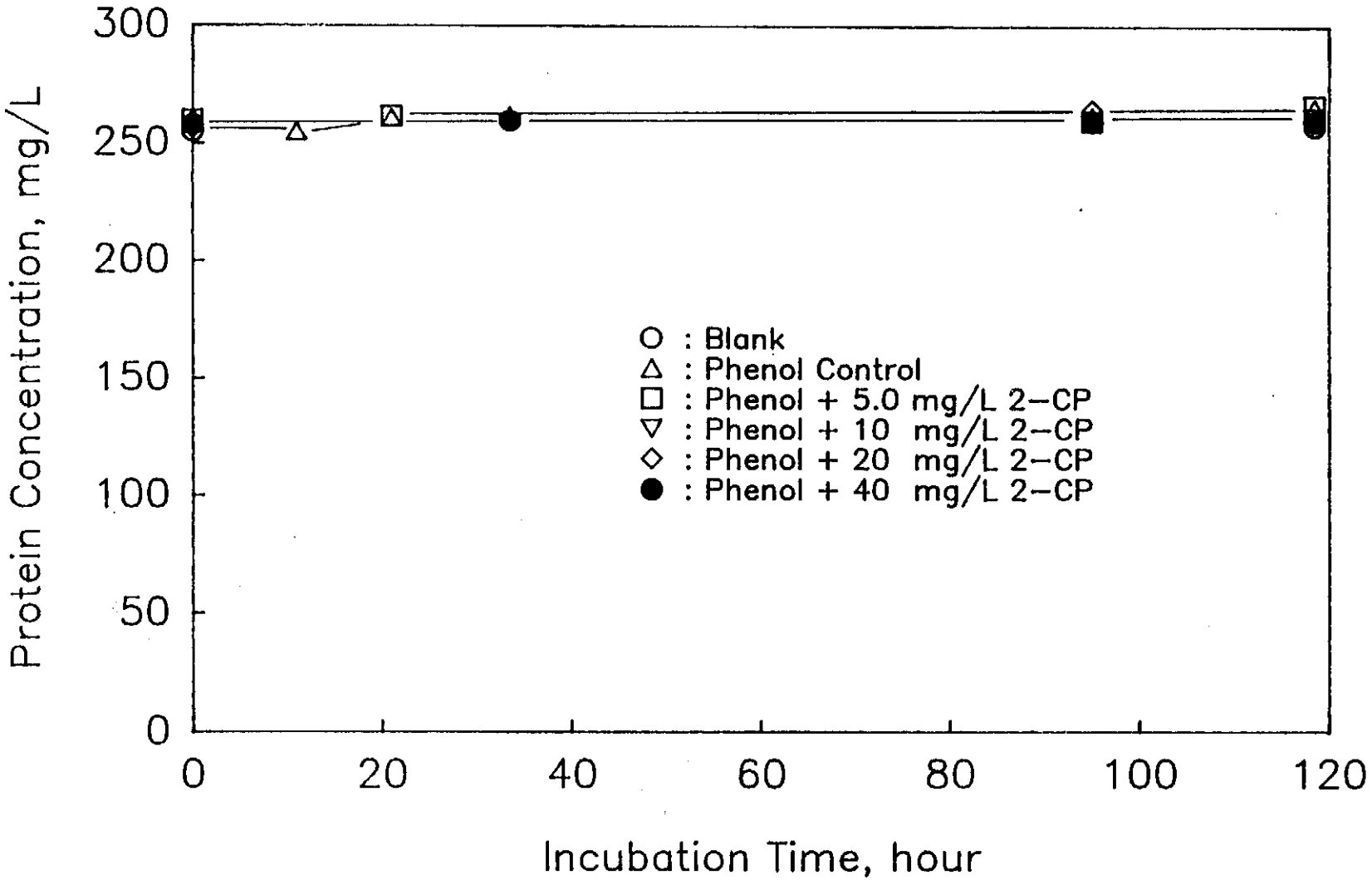

Figure 95. Growth of Biomass in the Phenol-supplemented Culture in the Presence of 2-CP: Early Stages

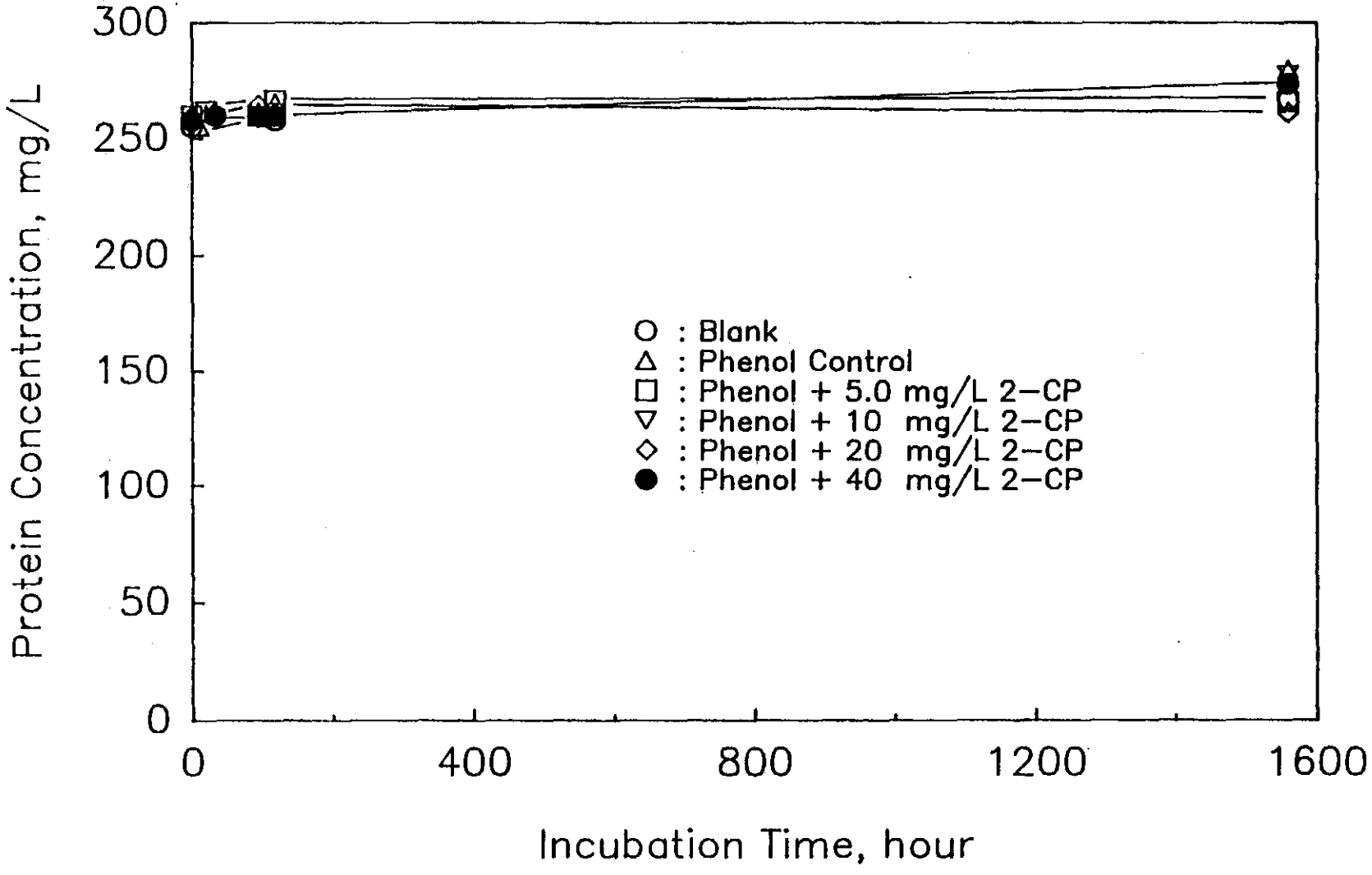

Figure 96. Growth of Biomass in the Phenol-supplemented Culture in the Presence of 2-CP: Entire Incubation

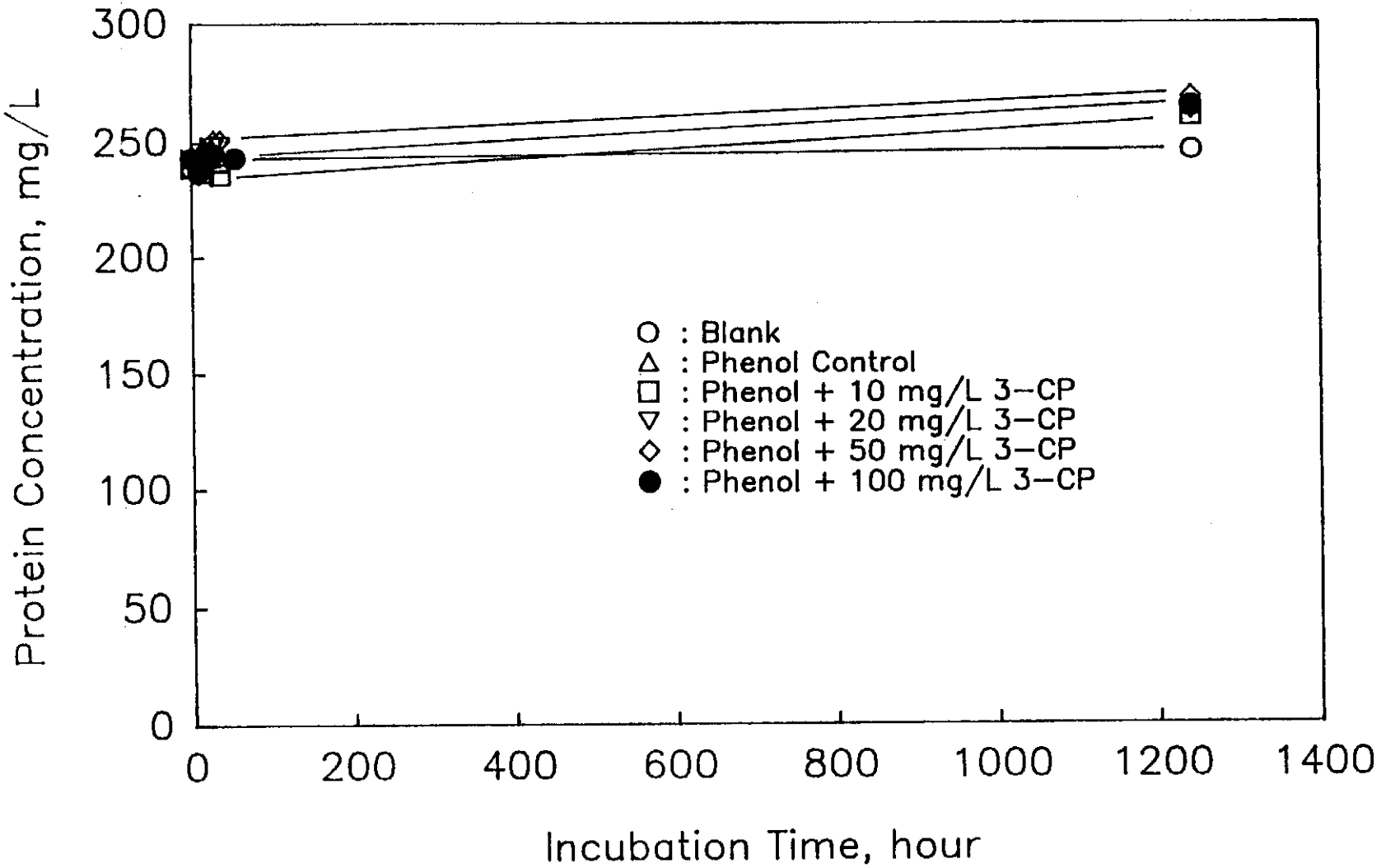

Figure 97. Growth of Biomass in the Phenol-supplemented Culture in the Presence of 3-CP

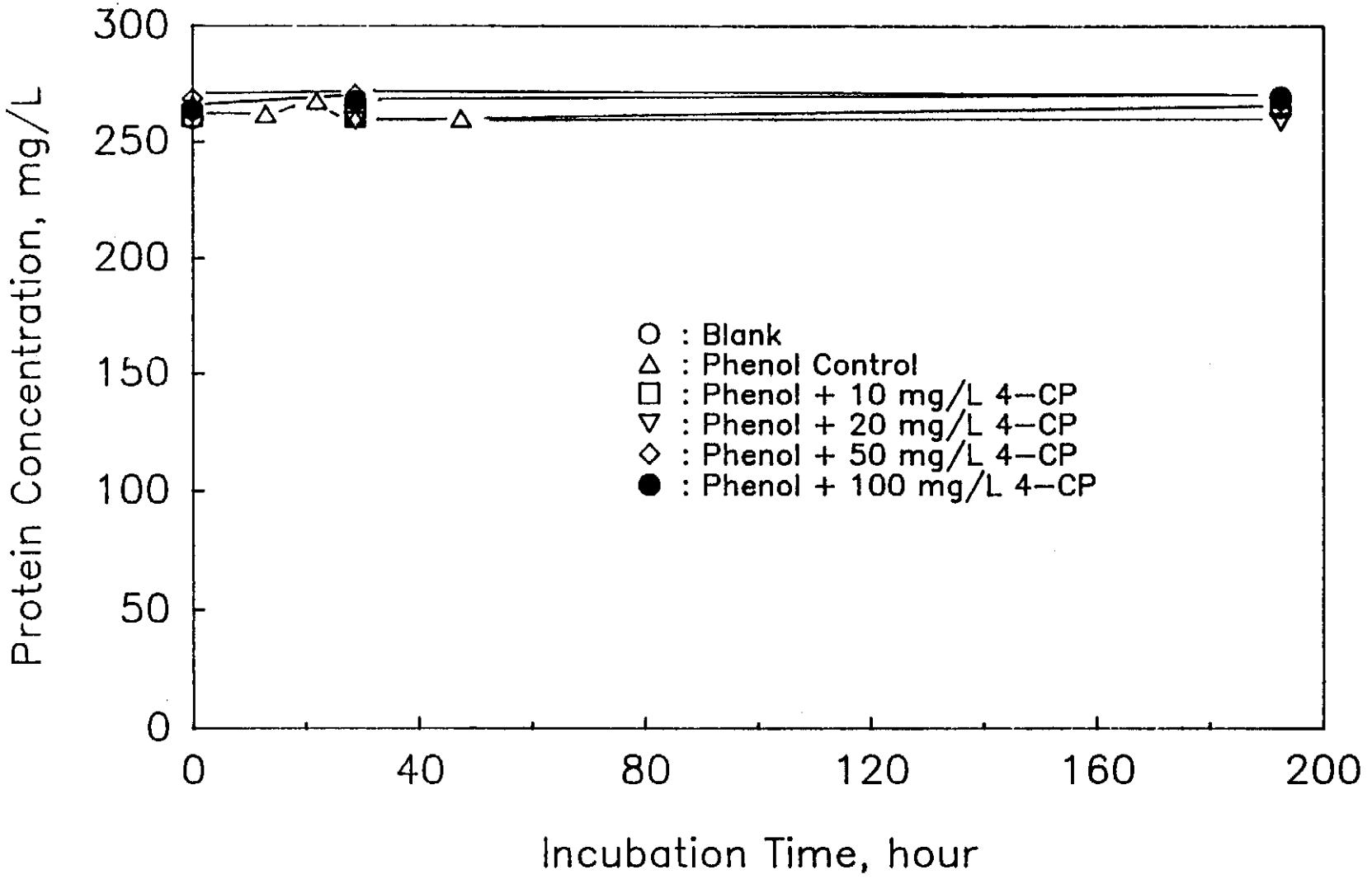

Figure 98. Growth of Biomass in the Phenol-supplemented Culture in the Presence of 4-CP: Early Stages

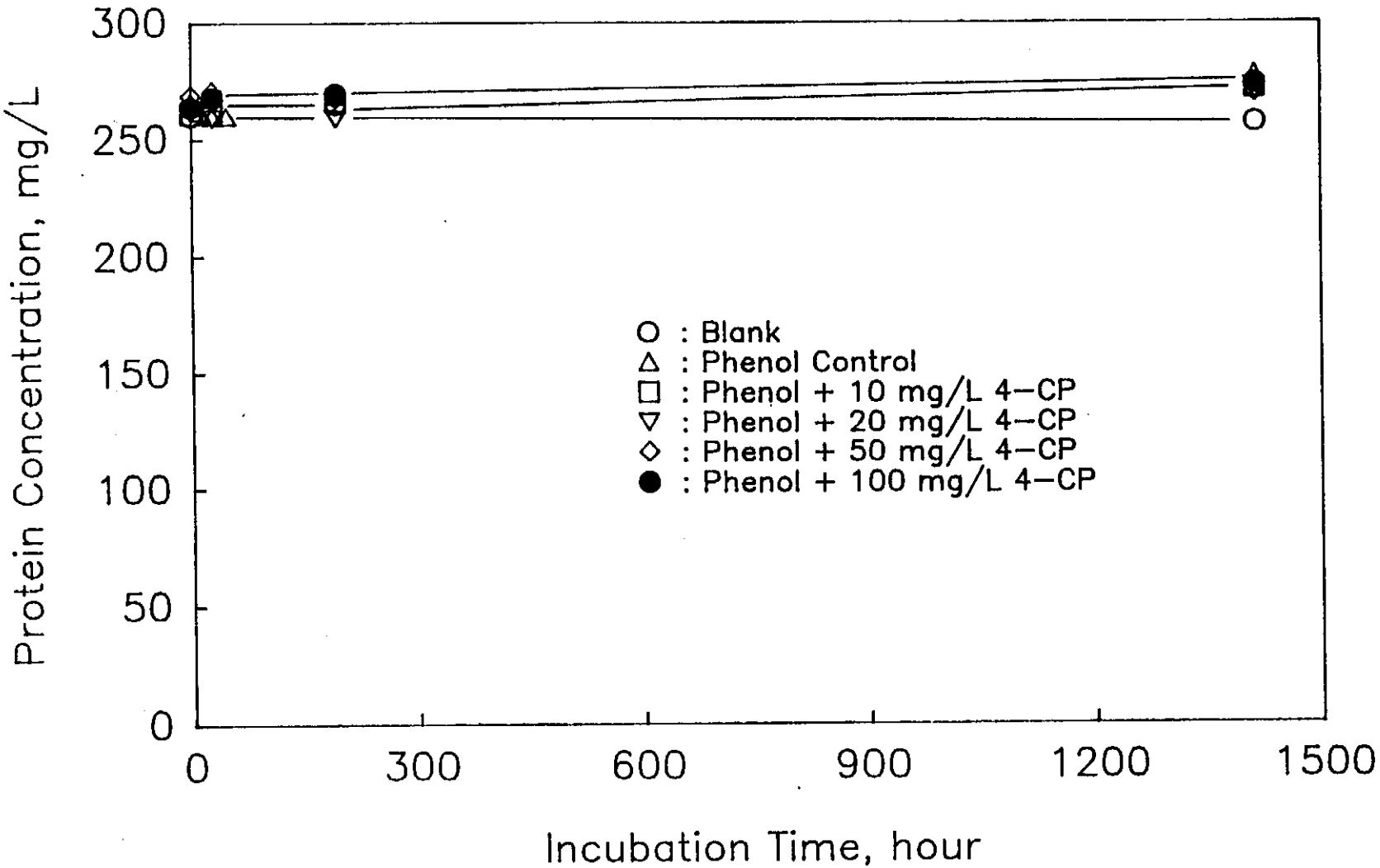

Figure 99. Growth of Biomass in the Phenol-supplemented Culture in the Presence of 4-CP: Entire Incubation

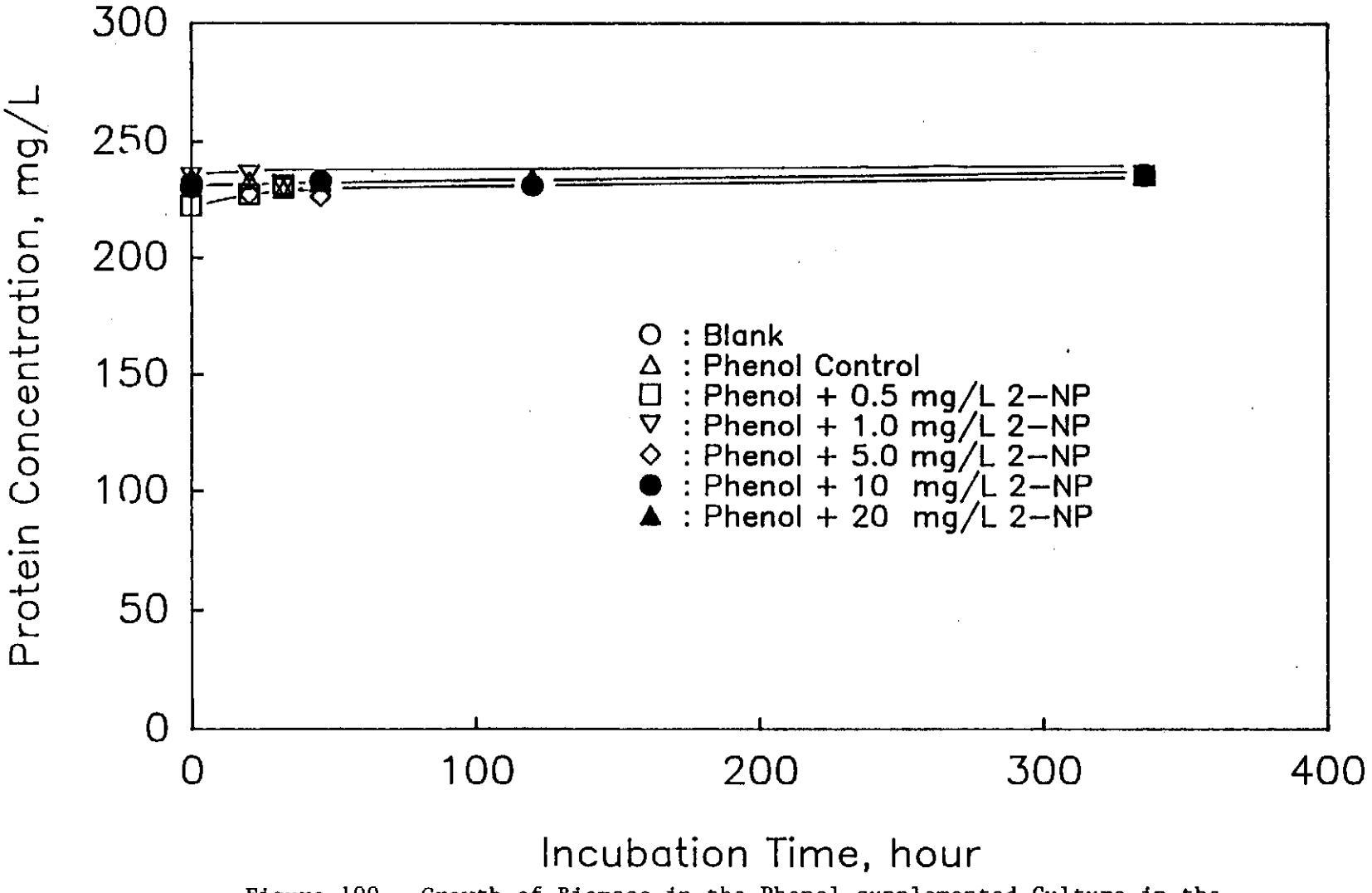

Figure 100. Growth of Biomass in the Phenol-supplemented Culture in the Presence of 2-NP: Early Stages

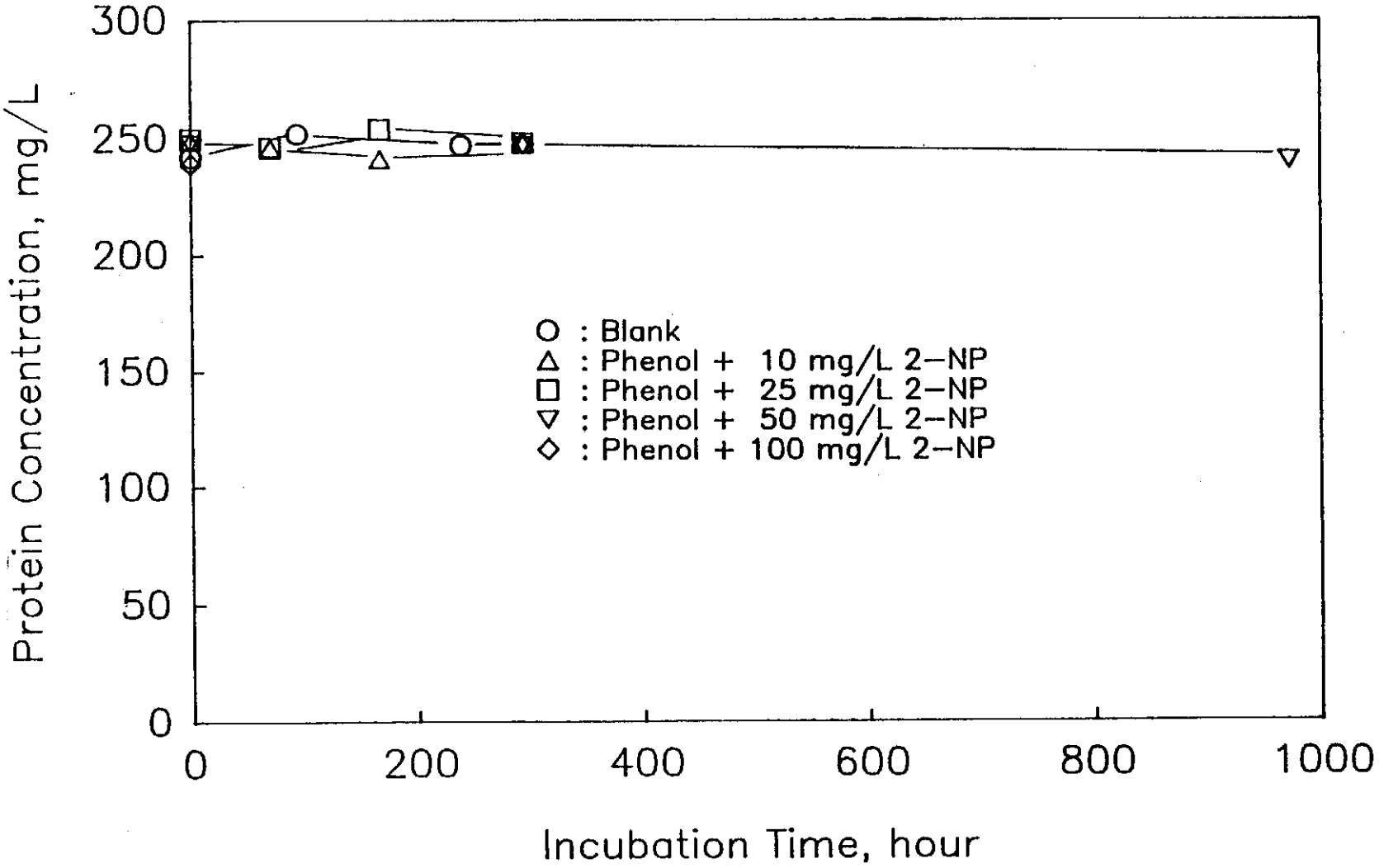

Figure 101. Growth of Biomass in the Phenol-supplemented Culture in the Presence of 2-NP: Entire Incubation

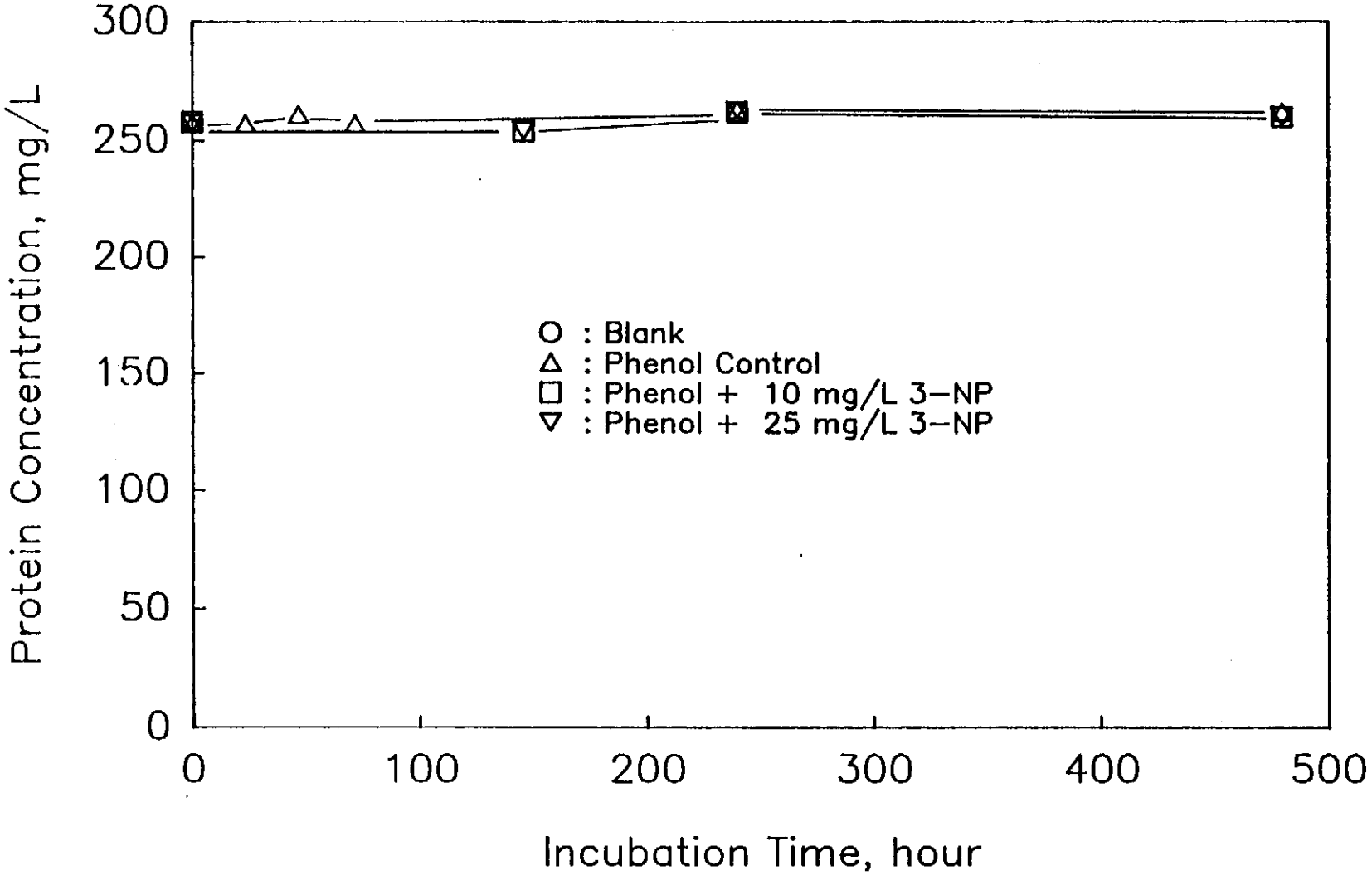

Figure 102. Growth of Biomass in the Phenol-supplemented Culture in the Presence of 3-NP

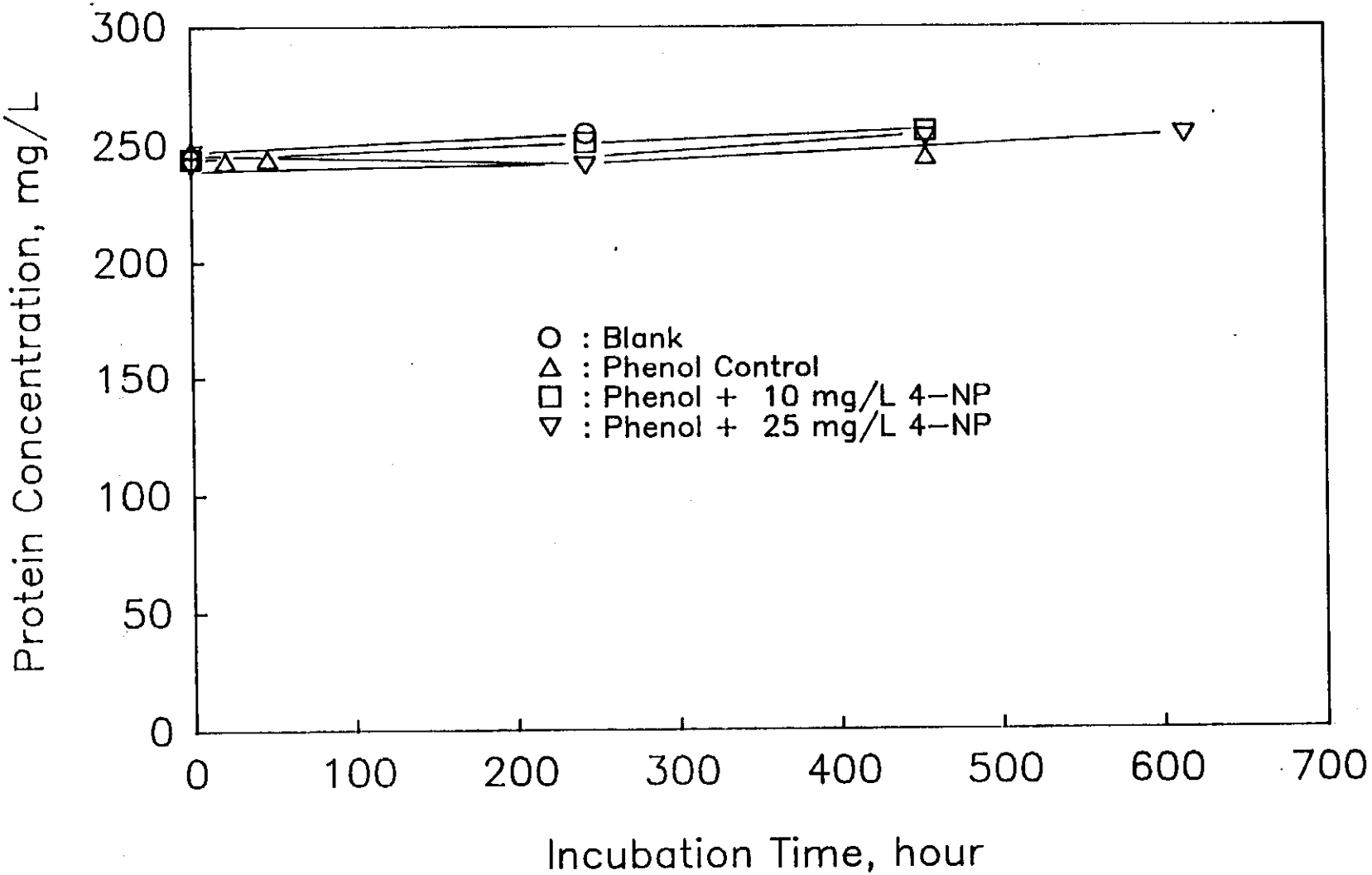

Figure 103. Growth of Biomass in the Phenol-supplemented Culture in the Presence of 4-NP

$$\frac{\partial t}{\partial K_I} = -\frac{1}{2K_I^2 X_o k}(S_o^2 - S^2) \quad (3)$$

$$\frac{\partial t}{\partial k} = -\frac{1}{k^2 X_o}\left(K_s \ln(S_o/S) + (S_o - S) + \frac{1}{2K_I}(S_o^2 - S^2)\right) \quad (4)$$

The Marquardt method of nonlinear regression was used to produce least-square estimates of the model parameters with an IBM 3090-300E supercomputer and Statistical Analysis System Computer Programs (SAS). Trial values of parameters were initially guessed and the model was solved and the model solution was compared to experimental data collected from the batch experiments. Equations 2-4 were used in the iteration to search for the set of kinetic parameters that best fit the experimental data by evaluating the residual sum of squares at each combination of values to determine the best set of parameter values.

The search for the best fit parameters was conducted for all data sets from a wide range of initial concentrations. Table 15 lists the biokinetic parameters which result in the least error between model solutions and experimental data along with kinetic constants reported earlier under methanogenic conditions. Parameters k and K_I found in this study were rather consistent with the previous findings. However, significant variations in K_s were noted. Further work may be needed to accurately determine the K_s value since the Haldane model exhibited no sensitivity to this parameter.

Using parameters listed in Table 15, the model describes both the phenol degradation and the catechol degradation rather well for lower initial concentrations (Figures 104-107). However, the Haldane model did not describe

Table 15 Summary of Kinetic Constants for Phenol and Catechol Methanogenesis

Compound	k, mg/mg protein-day	K_s, mg/ℓ	K_I, mg/ℓ	Source
Phenol	0.025	3.06	2163	this study
	0.028	0.03	363	Suidan et al (1988)
	0.003	700	966	Neufeld, et al (1980)
	0.014	46-90	900	Dwyer et al (1986)
Catechol	0.007	0.86	425	this study

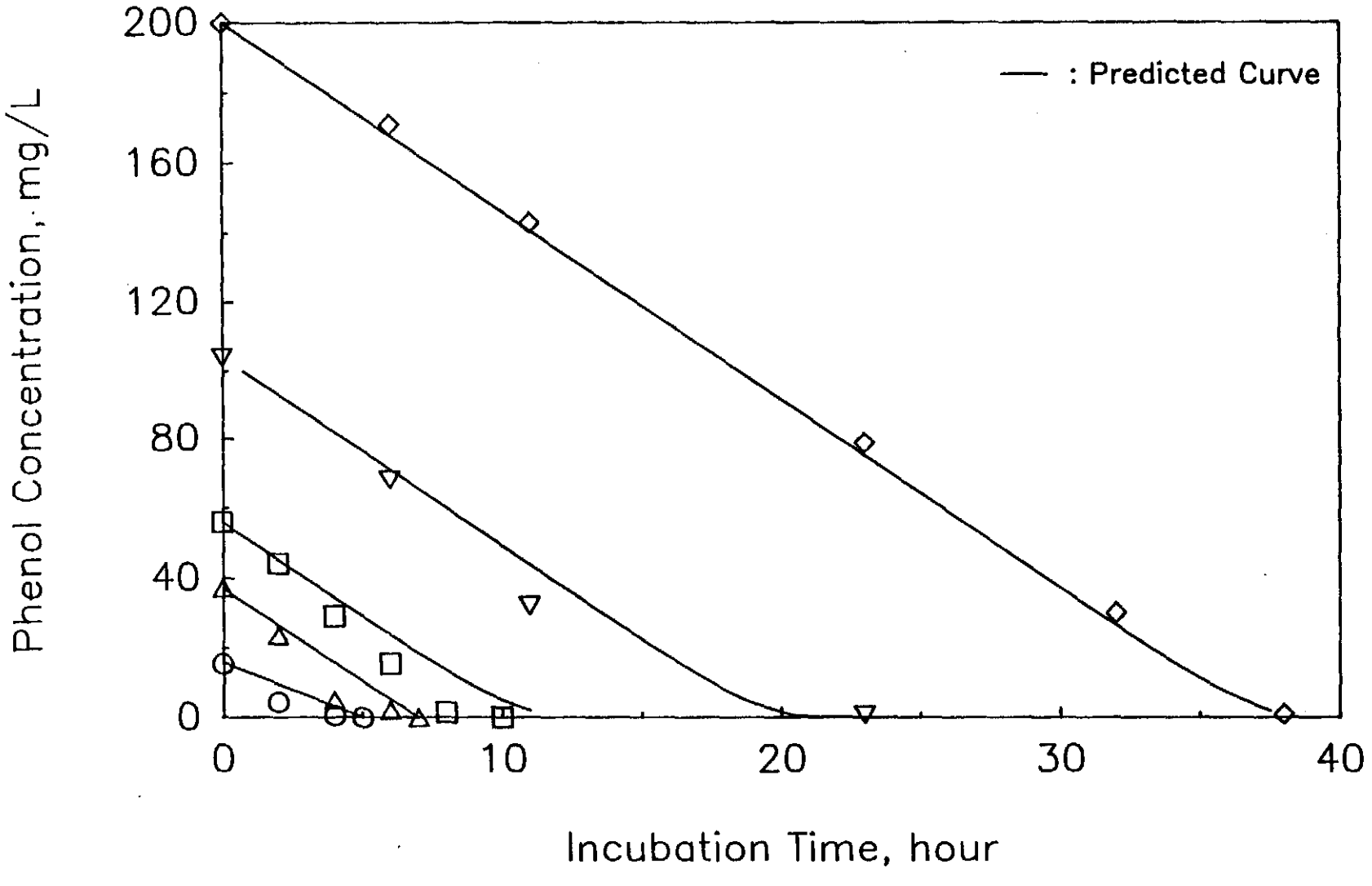

Figure 104. Haldane Inhibition Model Fit for the Anaerobic Biodegradation of Phenol: Lower Concentrations

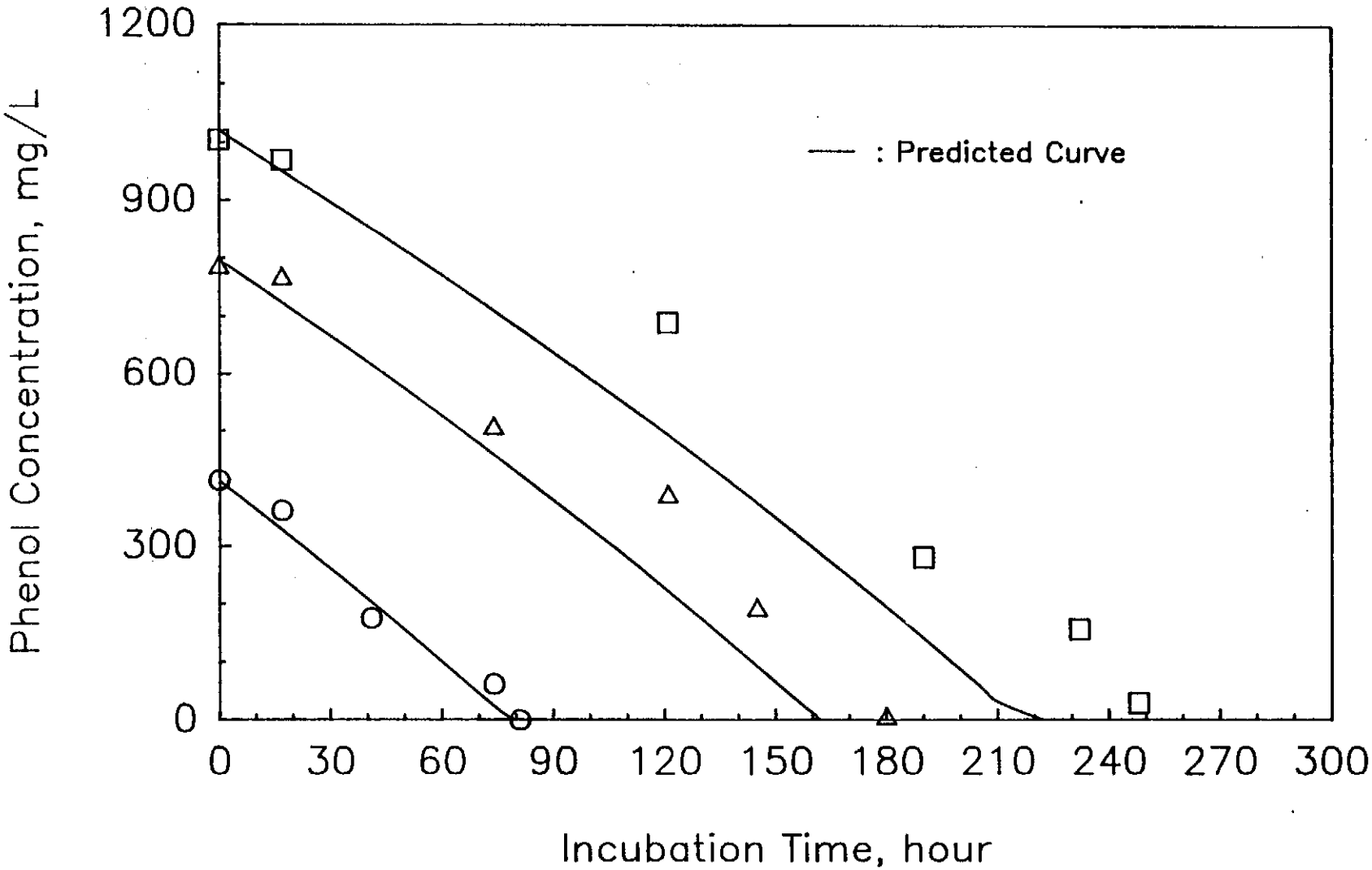

Figure 105. Haldane Inhibition Model Fit for the Anaerobic Biodegradation of Phenol: Higher Concentrations

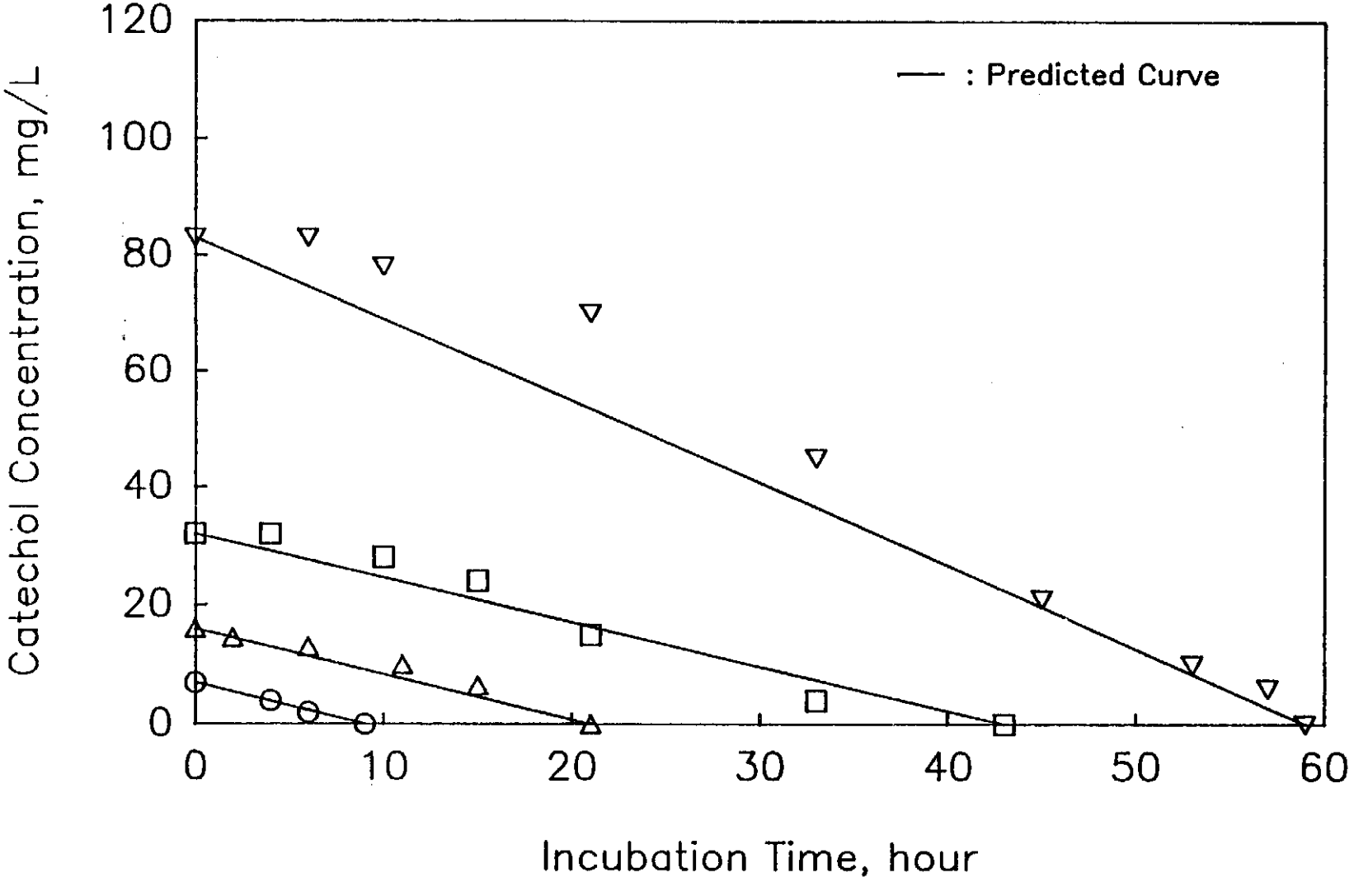

Figure 106. Haldane Inhibition Model Fit for the Anaerobic Biodegradation of Catechol: Lower Concentrations

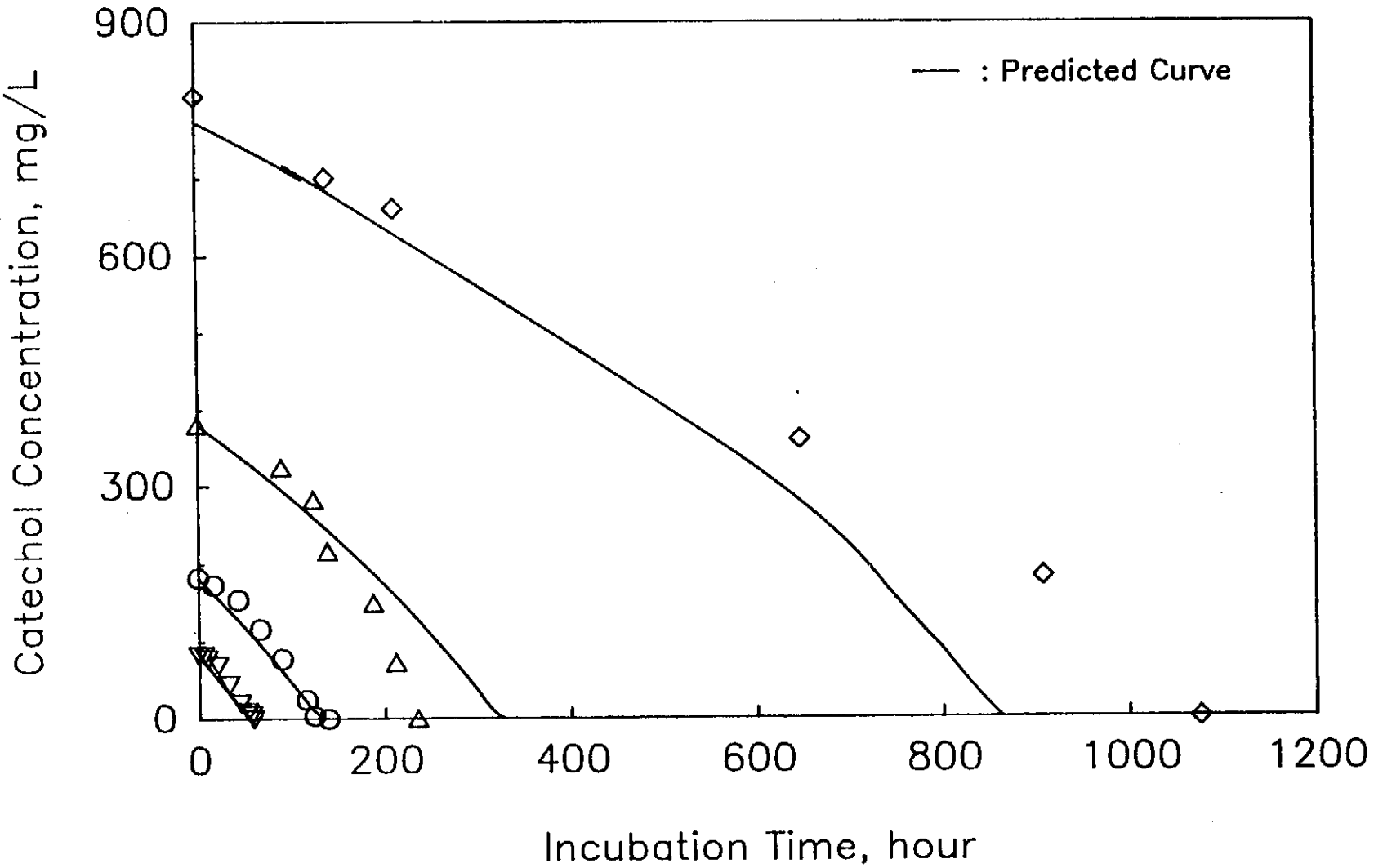

Figure 107. Haldane Inhibition Model Fit for the Anaerobic Biodegradation of Catechol: Higher Concentrations

the initial lag period noted for higher concentrations and therefore the model overestimated the rate of phenol degradation for higher concentration levels.

Inhibition of phenol degradation. The inhibition of phenol degradation by a substituted phenol was evaluated by fitting the batch data to each of the following three types of reversible inhibition models using the numerical technique developed for substrate inhibition:

Competitive:

$$-\frac{dS}{dt} = \frac{kSX}{K_s(1+T/K_I)+S} \tag{5}$$

$$\frac{\partial t}{\partial K_s} = \frac{1}{kX_o}(\ln (S/S_o))(1+ T/K_I) \tag{6}$$

$$\frac{\partial t}{\partial k} = \frac{1}{k^2 S_o}(K_s \ln(S/S_o) + (S-S_o) + K_s T/K_I(\ln(S/S_o) \tag{7}$$

$$\frac{\partial t}{\partial K_I} = \frac{K_s T}{kX_o K_I} \ln(S/S_o) \tag{8}$$

Uncompetitive:

$$-\frac{dS}{dt} = \frac{kSX}{K_s+S(1+T/K_I)} \tag{9}$$

$$\frac{\partial t}{\partial K_s} = -\frac{1}{kX_o}\ln(S/S_o) \tag{10}$$

$$\frac{\partial t}{\partial k} = \frac{1}{k^2X_o}\left(K_s\ln(S/S_o) + (1 + T/K_I)(S-S_o)\right) \tag{11}$$

$$\frac{\partial t}{\partial K_I} = \frac{T}{K_I{}^2kX_o}(S - S_o) \tag{12}$$

Noncompetitive:

$$-\frac{dS}{dt} = \frac{kSX}{(K_s+S)(1+T/K_I)} \tag{13}$$

$$\frac{\partial t}{\partial k} = -\frac{(1+T/K_I)}{kX_o}\ln(S/S_o) \tag{14}$$

$$\frac{\partial t}{\partial k} = \frac{1}{k^2X_o}\left(K_s(1+T/K_I)\ln(S/S_o) + (1 + T/K_I)(S-S_o)\right) \tag{15}$$

$$\frac{\partial t}{\partial K_I} = \frac{1}{K_I{}^2kX_o}\left(K_s\ln(S/S_o) + (S-S_o)\right) \tag{16}$$

Where T is the inhibitor concentration and all other parameters are as previously defined. The noncompetitive model was found to fit the data better than the other two models for inhibition of phenol degradation by resorcinol, hydroquinone, 2-CP, 3-CP, or 4-CP. Figures 108-112 show the model fit to

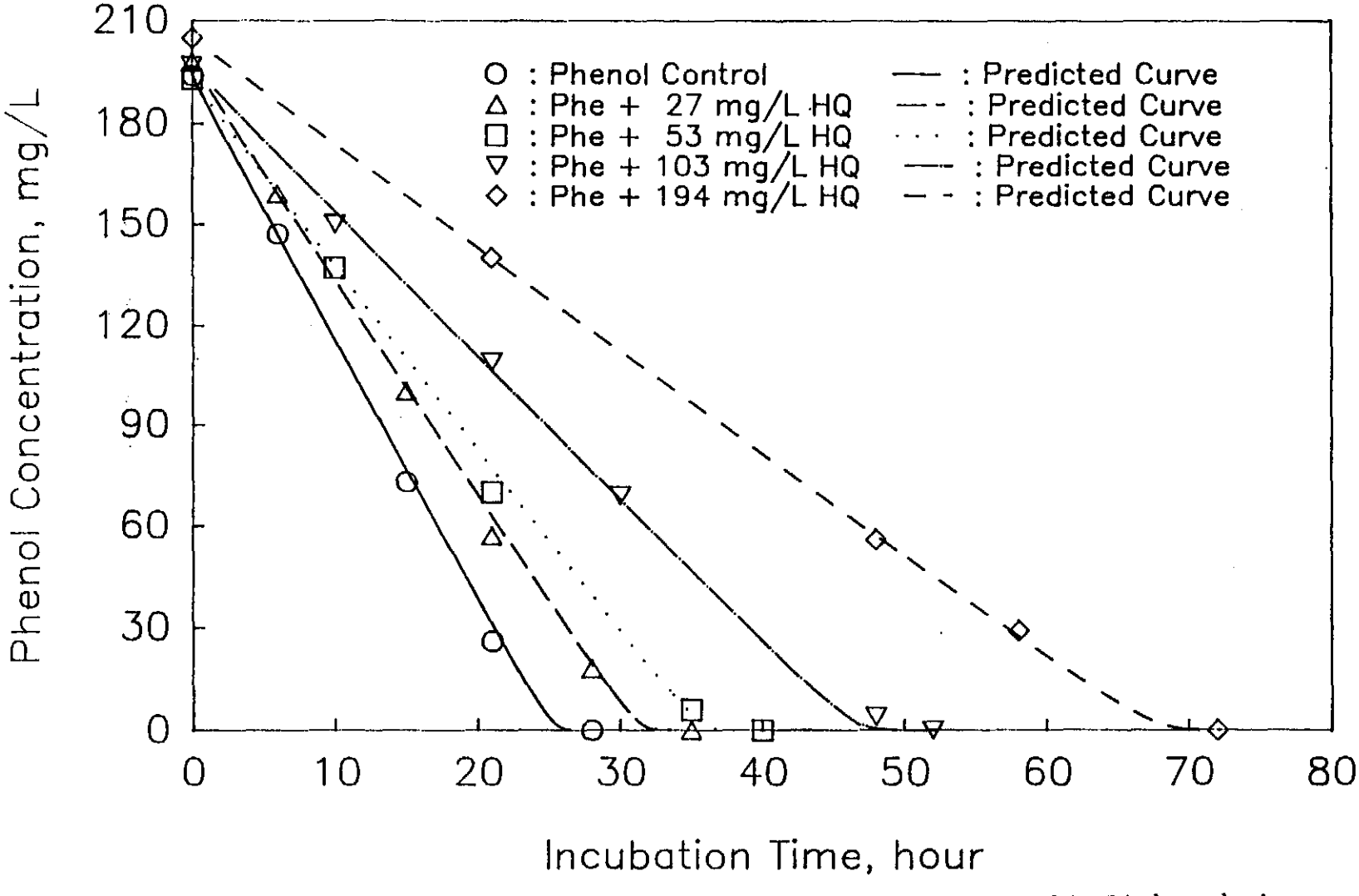

Figure 108. Noncompetitive Inhibition Model Fit for the Anaerobic Biodegradation of Phenol: In the Presence of Hydroquinone

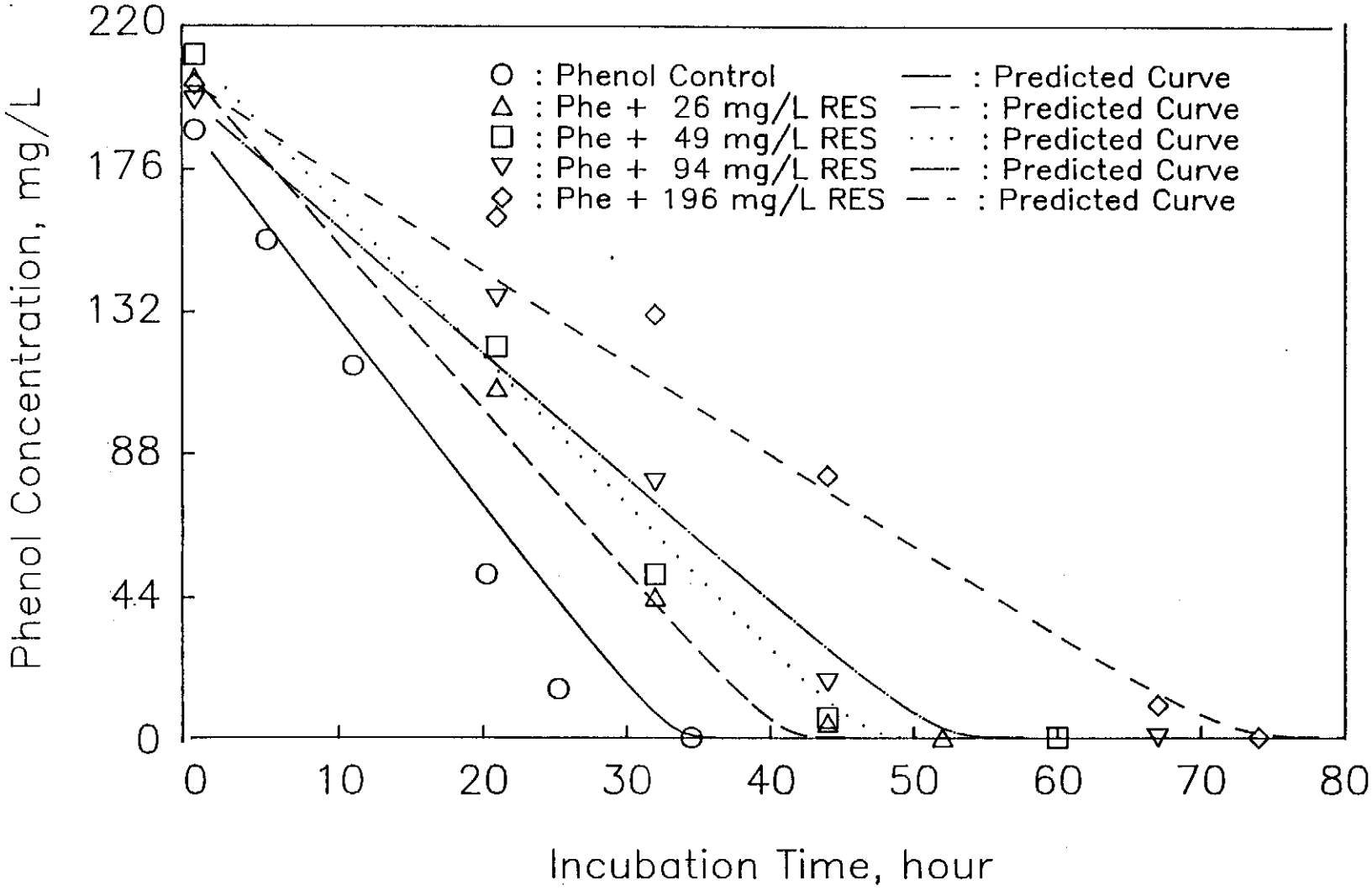

Figure 109. Noncompetitive Inhibition Model Fit for the Anaerobic Biodegradation of Phenol: In the Presence of Resorcinol

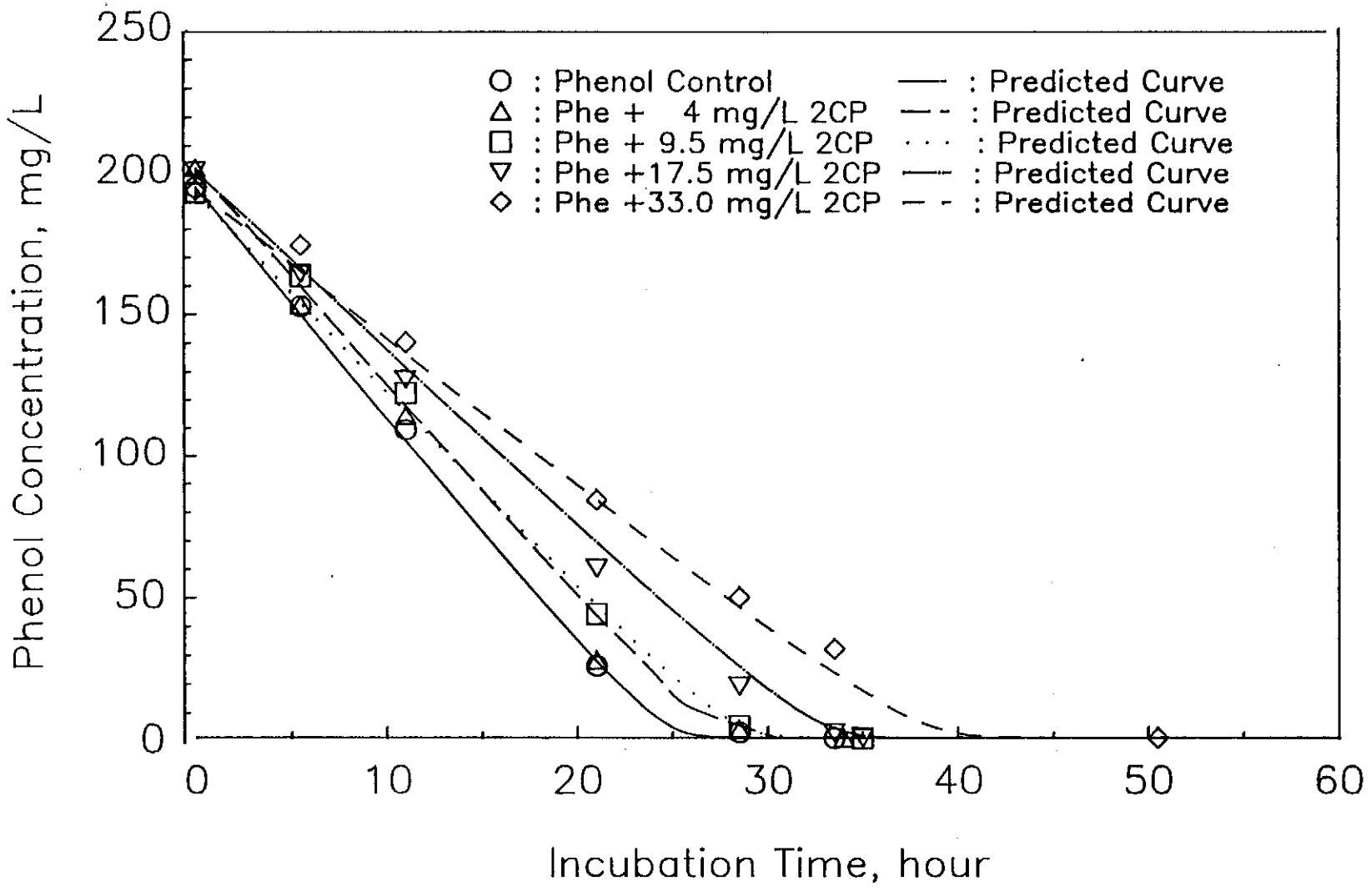

Figure 110. Noncompetitive Inhibition Model Fit for the Anaerobic Biodegradation of Phenol: In the Presence of 2-CP

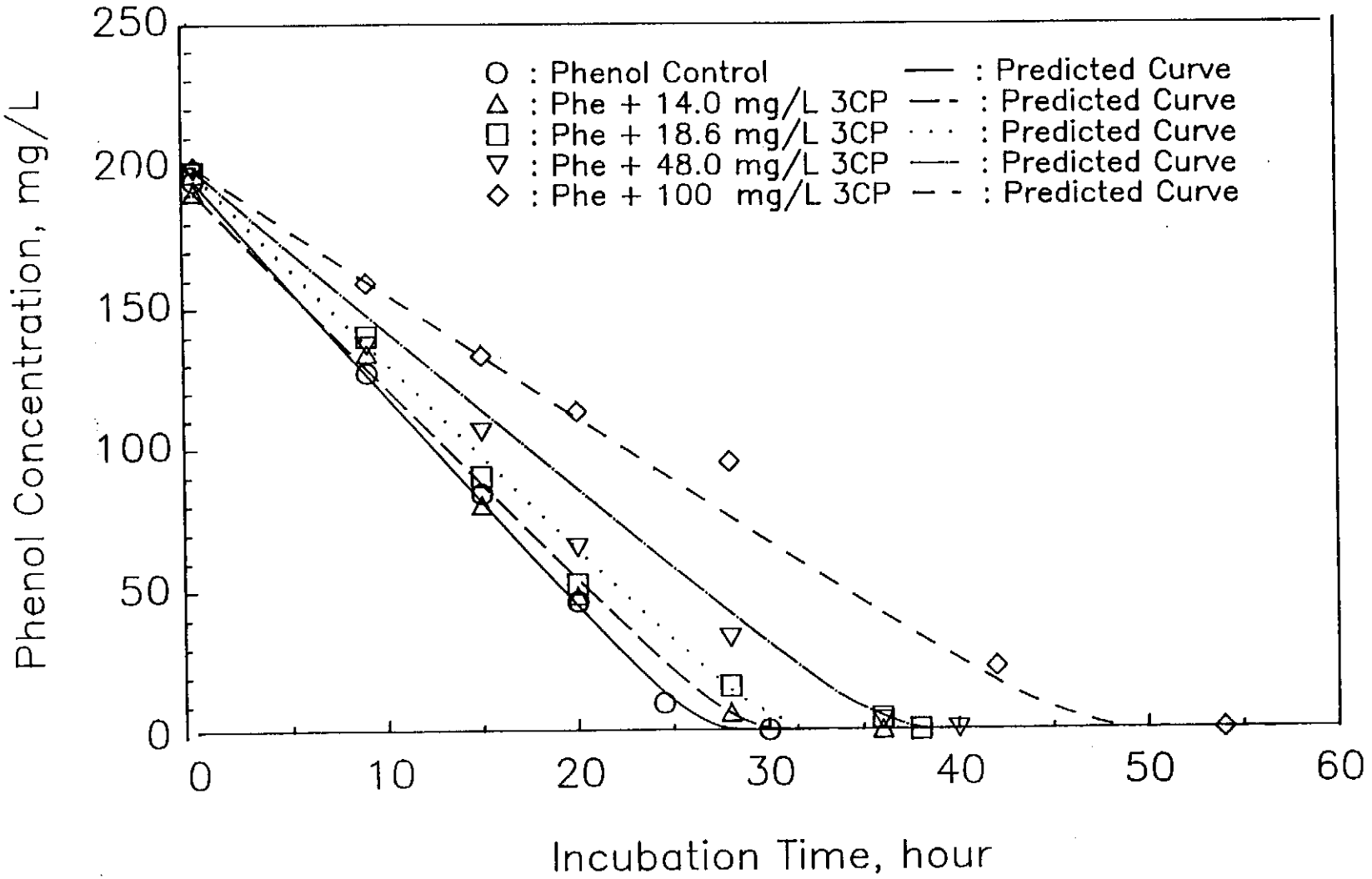

Figure 111. Noncompetitive Inhibition Model Fit for the Anaerobic Biodegradation of Phenol: In the Presence of 3-CP

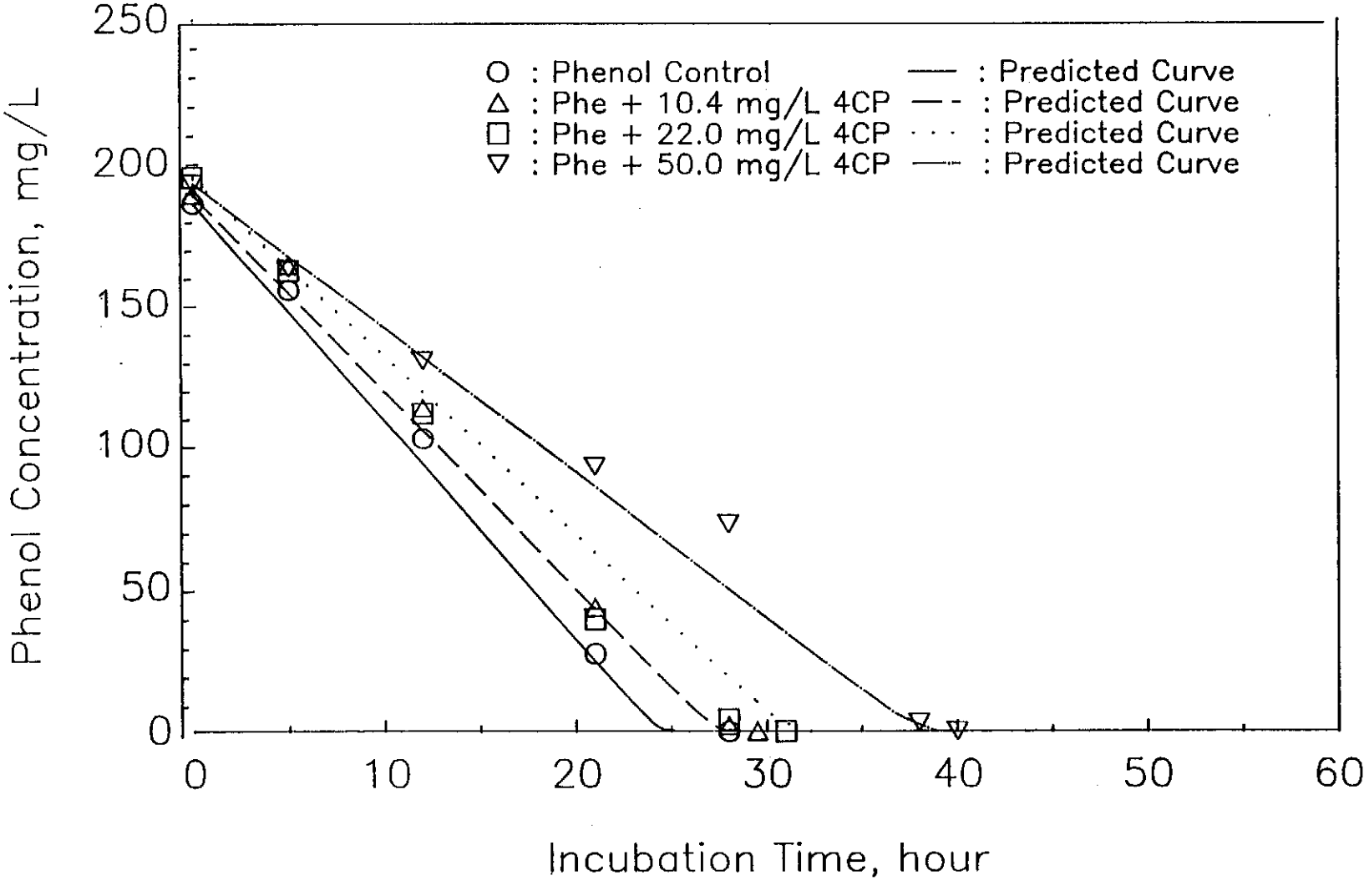

Figure 112. Noncompetitive Inhibition Model Fit for the Anaerobic Biodegradation of Phenol: In the Presence of 4-CP

experimental data using noncompetitive model and kinetic parameters listed in Table 16. In general, the model described the batch data well for a wide range of inhibition concentrations. However, the model overestimated the rate of phenol degradation under severe inhibition by higher inhibitor concentrations. The parameter k of 0.03 mg phenol/mg biomass protein/day was virtually the same as that obtained with the substrate inhibition model shown in Table 15. The value of inhibition constant K_I indicates the degree of inhibition. Lower values of K_I indicate stronger inhibition. Among the five compounds listed in Table 16, 2-CP had the lowest K_I value and therefore was the most inhibitory.

None of the three models described phenol inhibition well in the presence of catechol, 2-NP, 3-NP, or 4-NP. Catechol was degraded in the mixture over a wide range of concentrations (Figures 57-61) while nitrophenols were also disappeared (Figures 83-91), their inhibitory effects on phenol utilization can no longer be described by equation 5, 9, or 13 in which T was a constant. In addition, the model was not sensitive to K_s. Other approaches are needed to accurately determine the K_s value.

Table 16 Kinetic Constants of Noncompetitive Inhibition Model

Inhibitor	Phenol Substrate		
	k, mg/mg Protein-day	Ks, mg/ℓ	Ki, mg/ℓ
Resorcinol	0.025	3.39	191
Hydroquinone	0.03	2.18	127
2-CP	0.03	5.52	59
3-CP	0.03	4.16	141
4-CP	0.03	1.15	99

IV. CONCLUSIONS

Based on the findings of this study, the following conclusions can be drawn:

1. Benzene, toluene, and all three xylene isomers were not degraded to methane in the phenol-enriched culture even at the lowest tested concentration level of 10 mg/L.

2. Phenol and hydroquinone at concentrations of 200 mg/L or lower were partially degraded to methane in the acetate-enriched culture after two months incubation. Catechol and the three isomers of chlorophenol and nitrophenol were not degraded in the acetate-enriched culture.

3. Chlorophenols were persistent in the phenol-enriched culture. After a prolonged incubation period of more than eight months, the highest concentration degraded was only 5 mg/L.

4. All three nitrophenol isomers disappeared in the phenol-enriched culture at concentrations as high to 100 mg/L over a two-month incubation period. However, the nitrophenols were probably converted to methane only at concentrations of 30 mg/L or lower.

5. Hydroxyphenols were degraded to methane at much higher concentrations than chlorophenols and nitrophenols. Catechol and hydroquinone at concentrations as high to 800 and 1000 mg/L were converted to methane, respectively. However, resorcinol was degraded to methane only at concentrations of 100 mg/L or lower and at lower rates than nitrophenols.

6. The phenol-enriched culture was more susceptible to inhibition caused by substituted phenols than the acetate-enriched culture. The inhibitory effect toward both cultures was in the order of nitrophenols > chlorophenols > hydroxyphenols. However, the hydroxyphenols were less inhibitory than phenol toward the acetate culture. In general, the inhibitory effects on both cultures did not differ significantly among the isomer. The only exception

was found with catechol which was significantly less inhibitory than hydroquinone and resorcinol toward the phenol-enriched culture, suggesting that this culture was acclimated to catechol.

7. The Haldane inhibition model was used to fit the phenol and the catechol data and kinetic parameters that provided the best fit of the model to the experimental data were obtained. The model described substrate inhibition rather well with low and moderate initial concentrations. Model calculations deviated significantly from test data with higher initial substrate concentrations. In addition, the model exhibited no sensitivity to the Monod half velocity coefficient, K_s. Other approaches are therefore needed to accurately determine the K_s value.

8. The inhibition of phenol degradation by chlorophenols, resorcinol, and hydroquinone was described by a Monod-type, noncompetitive inhibition model. However, the model did not fit experimental data well with higher inhibitor concentrations. Other approaches are needed to describe the inhibitory effects observed with the biodegradable and/or biotransformable catechol and nitrophenols.

NOMENCLATURE

k: Maximum phenol utilization rate, T^{-1}

K_I: Haldane inhibition constant, ML^{-3}

K_s: Half velocity coefficient, ML^{-3}

S: Phenol concentration, ML^{-3}

S_o: Initial phenol concentration, ML^{-3}

T: Inhibitor concentration, ML^{-3}

X: Biomass concentration, ML^{-3}

X_o: Initial biomass concentration, ML^{-3}

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